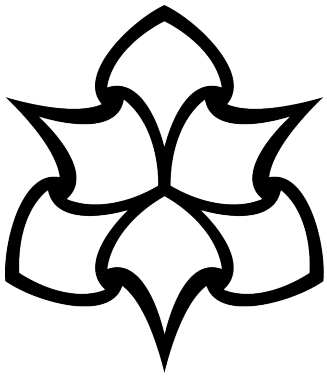

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LONGITUDINAL CHANGES IN MUSCLE AND MOBILITY IN SEPTUAGENARIAN MEN AND WOMEN

J A CAMERON

PhD 2019

Longitudinal changes in muscle and mobility in septuagenarian men and women

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A thesis submitted in partial fulfilment of the requirements of Manchester Metropolitan University for the degree of Doctor of Philosophy

Neuromuscular and Skeletal Ageing

Research Group

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Abstract

The effects of old age on musculoskeletal structure and function have been characterised in the literature through cross-sectional studies, comparing data from young and older adults in an attempt to uncover the mechanisms behind decreases in muscle mass and function. However, such work is prone to potential bias associated with differences in changes in lifestyle and genotype during human ageing. Longitudinal studies can overcome such bias, though these are of in the minority and generally outline relatively simple measures in larger epidemiological studies.

The overall aim of the work described in this thesis was to assess longitudinal changes in muscle mass and function, and how these influenced the ability to perform daily life activities over a 5-year period in older individuals. Characterising a more robust model of ageing than its cross-sectional counterparts in more detail than previously reported. This aim was addressed through several objectives that are described across the Chapters.

In Chapter 2, we highlight that the use of dual-energy X-ray absorptiometry (DXA) to track changes in muscle mass is a viable method when compared to magnetic resonance imaging (MRI), though DXA exhibited a positive intercept with MRI and therefore consistently overestimated muscle volume.

It was reported in Chapter 2 that over the 5-year period there might be an accelerated decline in muscle mass, with no difference in relative rate of muscle loss between genders or baseline muscle mass. Loss of muscle mass in ageing is related to deficits in functional capacity, in Chapter 3 the contribution of reduced voluntary activation,

fibre atrophy and fibre loss to muscle weakness are investigated. Muscle quality, measured by patella tendon specific force was found to contribute significantly to the loss of age-related muscle weakness in early ageing, though loss of muscle mass was found to be the main cause.

The objective of Chapter 4 was to investigate the influence of muscle weakness on mobility performance. Significant decreases in 6-min walk and timed up and go were noted, with changes in muscle power being the key contributor to this change. Rather than the intrinsic slowing of the muscle seen in early ageing, power loss was primarily due to reductions in maximal voluntary contraction.

The main conclusion of this work was that free-living septuagenarian's show an accelerated decline in muscle mass and functional performance over a 5-year period compared to cross sectional data previously reported, suggesting upon reaching the eighth decade humans neuromuscular system ages at an faster rate than preceding decades. The preferential atrophy of type II and loss of fibres are the key contributors to this loss of mass. These findings highlight the need for septuagenarians in relatively good health to have appropriate interventions designed to mitigate these age-related changes.

Acknowledgements

Firstly, I must note that this thesis is the combined effort of a group, where as an individual I was prodded, poked and sometimes forcibly pushed in the right direction. Over this journey there have been many ups and plenty downs and to this end I must thank my supervisory team for their constant drive and ability to guide me along this sometimes dark and long path. I am forever grateful to my Director of Studies, Prof Hans Degens, whose depth of knowledge and drive is unquestionable. I very much enjoyed our time together, with some of the best and worst jokes being told between us, isn't it? Is it? As well as our random WW2 chats, all conducted in a very gloomy (saving the world by not putting the lights on) office. Many thanks to Prof Jamie McPhee for your guidance on the subject in hand, alongside many of the issues you helped me deal with throughout the project, you have helped me more than you know and are just an all-round top bloke. I am thankful for the work you have both put into this project with me and what I have learned throughout the journey, I could not think of two better mentors for a PhD and most of all having excessive amounts of patience to deal with me for all this time. Without you both this project would never have happened.

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‘The Spirit that seeks to triumph in adversity and arms a man against the shock of battle is called Morale. The Moral of an individual or group is not necessity a measure of happiness or contentment; it is a measure of the cohesion and power of that individual’s or group’s resolve to pursue it object come what may.’

General Sir Rupert Smith

‘A moth-eaten rag on a worm eaten pole, It does not look likely to stir a man’s soul, ‘Tis the deeds that were done ‘neath the moth eaten rag, When the pole was a staff, and the rag was a flag.’

General Sir Edward Bruce Hamley

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List of Abbreviations

ACh: Acetyl choline

ACSA: Anatomical cross-sectional area

ALM: Appendicular lean mass

BC: Body composition

BIA: Bio-electrical impedance

BMD: Bone mineral density

BMI: Body mass index

CO: Cardiac output

CSA: Cross-sectional area

$CSA_{thigh:}$ Total cross-sectional area of the thigh musculature

CSA_{Quad}: Cross-sectional area of the quadriceps muscle

CT: Computer tomography

DXA: Dual-energy X-ray absorptimetry

FCSA: Fibre cross-sectional area

FFM: Fat free mass

FM: Fat mass

HR_{max}: Maximal heart rate

IGF-I: Insulin growth factor-I

IL-6: Interleukin 6

MAP: Mean arterial pressure

MCH: Myosin heavy chain

MRI: Magnetic resonance imaging

MVC: Maximum voluntary torque

PCSA: Physiological cross sectional area

PP: Pulse pressure

RF: Rectus femoris

R_{per}: Peripheral resistance

SV: Stroke volume

TUG: Timed-up-and-go

T tubules: transverse tubules

TNFα: Tumour necrosis factor α

QACSA: quadriceps anatomical cross-sectional area

VI: Vastus intermedius

VL: Vastus lateralis

VM: Vastus medialis

V_{CMJ}: Take-off velocity during the countermovement jump

6MWD: 6-minute walk distance

6MWT: 6-minute walk test

Chapter 1

General Introduction and Literature Review

1.1 General introduction

Skeletal muscle contributes around 40% of total body mass and serves out many different roles in the body, most notably the production of force which allows humans to execute movement, as well as thermoregulation, energy metabolism, endocrine and paracrine functions (Pedersen, 2009). As humans age, skeletal muscle mass declines progressively into later life. This process is known as muscle atrophy and it is a major cause of muscle weakness. This muscle weakness is associated with an impaired mobility that in turn has a negative impact on the quality of life and ultimately contributes to an inability to live independently (Goodpaster et al., 2006, Doherty, 2003a). The ageing-related degenerative loss of muscle mass and function has been termed Sarcopenia (Greek, meaning “poverty of the flesh”), however this umbrella term does not do justice to complex and multi-faceted nature of muscle related changes seen with ageing. Understanding this process is crucial as modern society has an increased proportion of older individuals, due to improved healthcare and living conditions.

The declining muscle mass with advancing older age is in part due to older people slowing down and moving less which causes a type of “disuse” atrophy. But, this is not the only cause because muscle mass, strength and other indices of muscular performance decline even in athletic older people from their fourth decade of life (Berthelot et al., 2012, Rittweger et al., 2009), which is very similar to the patterns seen in the general population (Janssen et al., 2000c). The declines are gradual, meaning that a substantial amount of muscle tissue and maximal function can be lost over several decades before it begins to interfere with a person’s ability to perform essential daily tasks. Ultimately, a “disability threshold” is crossed and beyond this the functional capacity of muscle function is impaired to such an extent that it impacts

negatively on daily life activities (Mithal et al., 2013, McPhee et al., 2016, Degens and McPhee, 2013). By this point, the risk of frailty, social isolation and morbidity are high (Jette and Jette, 1997). These issues highlight the importance of maintaining muscle force and velocity in old age to extend quality of life (Degens and Korhonen, 2012).

Much research has focused on cross-sectional changes in muscle function with age, which typically compares observations made in young adult with those made in older adults. Far fewer studies have assessed the longitudinal changes in muscle seen in ageing, and those studies that did determine longitudinal changes tend to be of epidemiology designs that cannot provide a detailed analysis. Longitudinal studies can circumvent bias related to genetic and/or environmental differences between people in cross-sectional studies.

1.2 Skeletal Muscle

Deliberate movement is a key function of skeletal muscle, alongside breathing and retaining posture (Pedersen, 2009). Skeletal muscle can be understood and studied in terms of its structure and function that enable force and power generation.

Structure

Skeletal muscle is made up of serial bundles of elongated, multinucleated cells also known as myofibres. The main proteins in muscle fibres are the two contractile proteins; actin (forming the thin filament) and myosin (forming the thick filament). The regular arrangement of the filaments along the length of the muscle fibre gives skeletal muscle a banded, or striated look (Jones and Round, 1990). Myosin can be broken down in to a globular head which combines with actin, the tail which combines with other myosin proteins to form thick filaments and the flexible region known as the S2 portion. Myosin filaments are attached to the Z line by the protein titin. Actin filaments

connect to the Z line structure via α-actinin. The other component of the thin filament is tropomyosin, which blocks the myosin binding sites until calcium binds to troponin C (Jones et al., 2004). These elements form the myofilaments, which are arranged in regular formations throughout the myofilament, constructing a series of sarcomeres.

Myofilaments are aligned serially to form a myofibril. Each myofibril is surrounded by transverse tubules (T tubules) and the sarcoplasmic reticulum, with around 2000 myofilaments forming a myofibril (Jones and Round, 1990). Myofibril then form muscle fibres, which aligned serially as bundles form the whole muscle, which connect via tendons to the bone. Each individual muscle fibre has one single connection and the neuromuscular junction to a motor neuron of the nervous system. Instructions transmitted along the nervous system to the muscle lead to muscle fibre contractions and thus force generation to cause movement of skeletal elements relative to each other.

Mechanics of contraction

The functional unit of skeletal muscle is the motor unit, which consists of the motor neuron in the spinal cord, its axon that extends towards the target muscle, and the muscle fibres it innervates (Sherrington, 1925). Each motor neuron innervates a number of muscle fibres of the same phenotype (slow or fast) distributed throughout the target muscle, ensuring even force distribution throughout the muscle and avoids localised intense activation (Edström and Larsson, 1987). The number of fibres innervated by each motor unit is dependent on the type of movement required (finer movement require smaller motor units, while large and powerful movements require large motor units) and the size of the muscle (Jones and Round, 1990). Where the motor neuron meets the muscle fibre there is a synaptic connection known as the

neuromuscular junction. When action potentials from the motor neuron reach the neuromuscular junction, the voltage sensitive channels open and calcium enters into the synapse. This in turn releases acetyl choline (ACh) into the synaptic cleft, increasing permeability to Na^+ of the postsynaptic membrane which leads to a rapid depolarisation of the muscle membrane. The action potential then propagates over the muscle membrane and reaches the interior of the fibres via the invaginations of the plasma membrane, called T tubules. Consequently ryanodine receptors (calcium channels) open on the sarcoplasmic reticulum following the conformation change of the voltage-gated dihydropiridine receptors (Huang et al., 2011). Calcium diffuses to the myofilaments, where it binds to troponin C and induces a conformational change in tropomyosin exposing the myosin binding sites. The myosin head then binds to actin, the bond is strengthened through the release of inorganic phosphate and during the release of adenosine di-phosphate the power stroke occurs, causing the filaments to 'slide' along each other, resulting in shortening of the muscle fibre and production of force. Myosin detaches from actin when adenosine tri-phosphate binds, which is then hydrolysed to adenosine di-phosphate and inorganic phosphate, returning the myosin head to its original position. This process is known as cross bridge cycle and the entire process is identified as excitation contraction coupling (Huxley, 1957).

Power Generation

When cross bridge cycling occurs, the muscle produces force and shortens (at a variable velocity), though isometric muscle contractions occur without appreciable shortening between insertion and origin. Force multiplied by velocity equals power, and this production of power allows movement of the skeletal system. This relationship is outlined by the Hill equation (Hill, 1938).

$$(P + a) * (V + b) = (P_0 + a) * b$$

where P_0 is the maximal isometric force, and a and b are constants, V the velocity of shortening as a proportion of the maximal shortening velocity (V_{max}) and P the force as a proportion of P_0. Therefore the product of force (N) and velocity ($m \cdot s^{-1}$) during contraction gives power ($N \cdot m \cdot s^{-1}$) (Degens, 2018). Production of muscle force is proportional to its cross-sectional area, while velocity of shortening is proportional to the length of the muscle (although differences occur across different fibre types). Therefore, a muscle that is thin and long will shorten quickly but yield low force, while the opposite is true for thick and short muscles (Jones et al., 2004).

Fibre Type

The fibre type composition is an important determinant of the activation time and relaxation time. There are three pure fibre types in human skeletal muscle: type I, type IIa and type IIx. Additionally, some fibres are hybrid fibres containing more than one myosin heavy chain (MHC) isoform. The difference in MHC isoforms defines the contractile properties of each fibre type (Harridge et al., 1996), though other contractile proteins, such as tropomyosin have also been shown to have fast and slow isoforms (Tajsharghi, 2008). Type IIa/IIx are both more powerful but fatigue more easily due to greater anaerobic respiration, while type I are more resistant to fatigue with the cost of being relatively slow (Jones et al., 2004). The maximal shortening velocity is as much as 7x higher in fast than slow fibres (Bottinelli et al., 1996, Widrick et al., 1996, Gilliver et al., 2009, Degens and Larsson, 2007).

Muscle architecture

Muscle architecture is key to the understanding of mechanical properties of muscle, giving further insight into the force-velocity and length force relationship. The

physiological cross-sectional area (PCSA) was first outlined by Haxton (1944) and is the cross section of the muscle perpendicular to its fibres, allowing an estimation of force proportional to the number of fibres. The addition of muscle and joint architecture allow the influence of external torque to be accounted for and allows for better estimates of muscle quality and is known as specific force, this then allows for the force velocity characteristics of the muscle to be obtained (Degens et al., 2009b).

1.3 Changes in muscle with age

The ageing process is associated with a general reduction of skeletal muscle mass, termed sarcopenia (Rosenberg, 1989a). The loss of muscle mass is associated with weakness, reduced mobility, balance and neuromuscular control, causing older individuals to suffer a decreased quality of life, progressing from an independent to dependant lifestyle (Russ et al., 2012). These consequences of muscle wasting can be detected even in healthy older people and can lead to a reduced quality of life (Doherty, 2003a) also manifesting itself with increased incidence in physical disability and mortality (Hairi et al., 2010a, Janssen et al., 2002).

As the percentage of people older than 65 years in Western societies continues to increase, it is becoming ever more important to design effective strategies to temper or even reverse the decline in muscle function associated with ageing. The much cited cost of this age related decline into disability is estimated to cost the United States health service around $18.5 billion in 2000 (Janssen et al., 2004). More recent work showed individuals defined with low skeletal muscle mass index consistently having higher associated costs in a wide range of settings whether these be direct or indirect healthcare costs (Norman and Otten, 2018). A cohort of older people (71-80 years) in the UK classified with muscle weakness was shown to cost £4592 per annum

compared to £1885 per annum in those without muscle weakness, therefore the financial cost to the UK is currently estimated at £2.5 billion (Pinedo-Villanueva et al., 2019). With the cost to the United States health service now estimated to be $40.4 billion (Goates et al., 2019). The global proportion of older individuals continues to grow, the financial burden will continue to rise as the direct and indirect health care costs of sarcopenia increase (United-Nations, 2012, McPhee et al., 2016, Norman and Otten, 2018). There are many factors that contribute to sarcopenia including altered hormonal status, inflammation, oxidative stress, altered protein intake and anabolic resistance, all have an effect on how humans age, leading to issues with these confounding factors particularly in cross-sectional studies (Narici and Maffulli, 2010, Degens, 2007, Larsson et al., 2019). There is evidence that the decrease in muscle function with age is proportionally more than the loss of muscle mass, leading to the suggestion that there is a lower 'quality' of remaining muscle tissue in old age (Degens et al., 2009a, Canepari et al., 2010, Morse et al., 2005a). We must also consider that those in old age represent many specific cohorts (good health/poor health), all with differing rates of ageing due to multiple factors and it is important that we are able to characterise such phenomena within these cohorts.

Age-related changes in Muscle Mass

Whole body muscle mass loss between the ages of 18 and 88 is around 27% (Janssen et al., 2000c). This loss of mass can mainly be attributed to a decline in numbers of muscle fibres and to atrophy of remaining fibres, particularly the type 2 fibres (Lexell et al., 1988). It has also been reported that muscle mass loss is not uniformly spread throughout the body, with the upper body seemingly less affected from losses than the leg muscles (Janssen et al., 2000c). It would also seem that the four muscles of the quadriceps are more affected than other muscles of the legs (Maden-Wilkinson et al.,

2013b, Abe et al., 2014b). In fact, extensor muscles in general seem to suffer from more atrophy than flexors (Candow and Chilibeck, 2005, Abe et al., 2014a), with the quadriceps possibly losing up to 40% of its volume by the ninth decade (Macaluso et al., 2002, Young et al., 1984). This loss of muscle mass seemingly starts after the age of 30 years (Gallagher et al., 1997) and constant progressive losses are seen when entering the sixth decade of life (Janssen et al., 2000c, Doherty, 2003a, Deschenes, 2004).

Men have larger muscles that produce higher forces than women (1.5-2 times higher) (Goodpaster et al., 2001, Miller et al., 1993), though whether the rate of atrophy is greater is up for debate with it thought to be similar (Frontera et al., 2000a, Koster et al., 2011) or faster in men (Delmonico et al., 2009a, Hughes et al., 2001b). While it is unequivocal that in absolute terms men's muscle mass declines at a faster rate, because of an initial larger muscle mass they will reach the disability threshold later than women will. Therefore, older women may be more susceptible to mobility issues and an increased likelihood of sarcopenia (Degens and McPhee, 2013, Narici and Maffulli, 2010).

When looking at the loss of muscle mass, there is a particular interest in the knee extensor muscles, due to their function in movement and functional limitation has been reported to be related with a small quadriceps mass (Buford et al., 2012). The use of Magnetic resonance imaging (MRI) allows for the thigh to be separated into its constituent muscles (Buford et al., 2012, Narici et al., 1992, Ogawa et al., 2012). MRI has allowed for differential changes in these muscles to be observed, such as a faster rate of atrophy of the quadriceps muscle with a relative maintenance of the hamstrings and adductors muscle mass during ageing (Janssen et al., 2000c, Macaluso et al., 2002, Ogawa et al., 2012)

Power loss

It seems that the age-related changes in muscle power begin earlier than the observed changes in muscle mass. When power decreases below a certain level it will impact on the ability of to perform tasks of daily life (McPhee et al., 2016, Degens and McPhee, 2013) and even in healthy older people. Reductions in chair rise time and walking speed are reported, that can partly be attributed to a loss of muscle power but not loss of muscle mass (Maden-Wilkinson et al., 2015). The rate of loss of power appears similar in men and women (Maden-Wilkinson et al., 2015, Runge et al., 2004).

Figure 1.1 illustrates how age-related decrements in force and velocity both contribute to the loss of power with age. Since both force and velocity determine the power generated, it is understandable that muscle power is more strongly correlated with performance of daily life activities than muscle force or mass (Maden-Wilkinson et al., 2015, Reid and Fielding, 2012). Whether these observations actually precede detectable changes in electrophysiological or morphological measures is debatable, as these may be missed due to lack of sensitivity and precision in these measures at this point (Larsson et al., 2019).

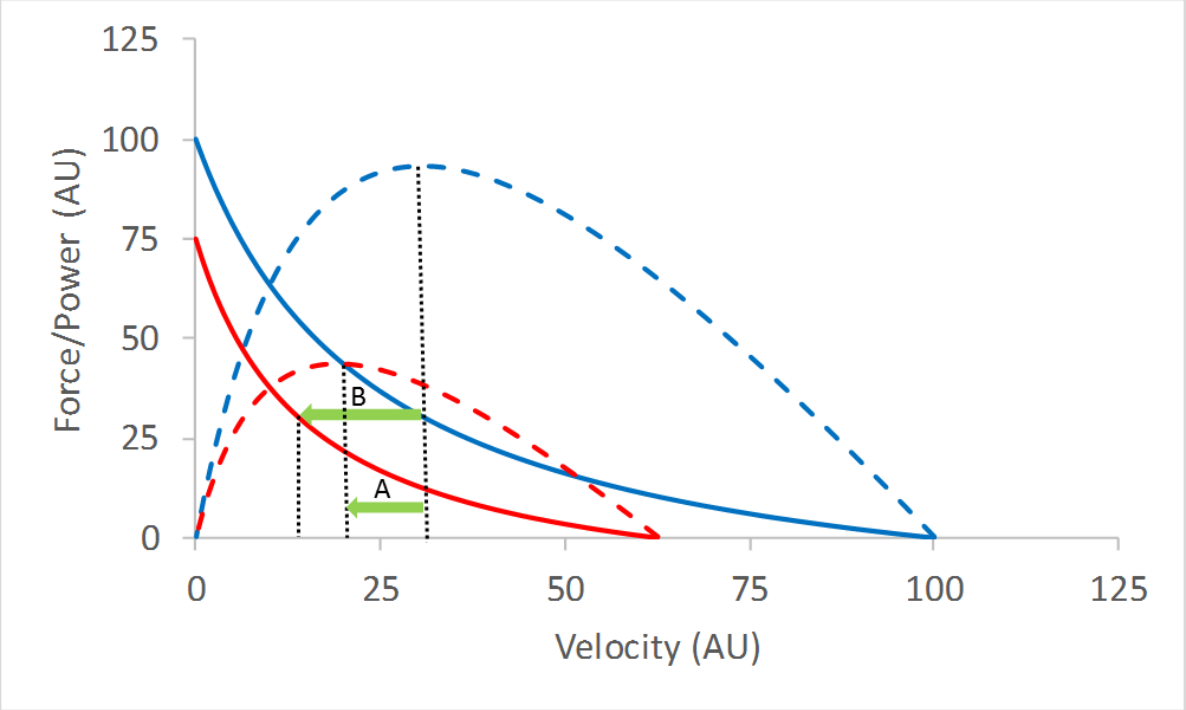

Figure 1.1. Illustration of the force (solid lines) and power (dashed lines) velocity relationships in young (blue lines) and old (red lines) muscles, where the maximal velocity of shortening and force are set at 100% in young muscles. For the illustration, a 25% lower maximal force and a 40% lower maximal shortening velocity in old than young muscles are assumed, while the curvature is kept constant. This resulted in a 53% lower maximal power and a lower velocity at which maximal power is generated, illustrated by arrow A. Arrow B illustrates that to generate the same force, older muscles must contract slower. Both effects most likely contribute to some extent, but not solely, to the slowing of movement in old age (Degens, 2018).

Force, power and velocity of shortening decrease with age (Edstrom and Larsson, 1987, Degens et al., 1998, Cotter et al., 1989), independent of protein composition of the fibre (Degens and Larsson, 2007). However, it has been shown that there is a disparate change in muscle force and power generating capacity on the one hand and size on the other hand, suggesting that not only do muscles atrophy but the contractile properties are also compromised (Degens et al., 2009b).

Fibre type and size

It is generally considered that there is no shift in numerical muscle fibre proportions with ageing, but that there is increased areal proportion of type I fibres, due to preferential atrophy of type II fibres (Andersen, 2003, Barnouin et al., 2017b). This

preferential type II atrophy also leads to whole muscle atrophy and will undoubtedly also contribute to the age-related loss of power, as type II fibres are more powerful than type I fibres (Gilliver et al., 2009). However, the decrease in the areal proportion of type II fibres (e.g. from 57.5 at 24 years to 51.5% at 70 years) is not large (Barnouin et al., 2017b) and the changes in fibre type composition thus contributes a small part to the loss of muscle power seen with ageing (Degens, 2018).

Architecture

During ageing the muscle architecture changes as a consequence of the muscle fibre atrophy and loss of fibres. The most obvious consequence is a reduction in the cross-sectional area of the muscle, but also the pennation angle of the fascicles decreases. This reduction in pennation angle will attenuate some of the age-related reduction in force and power generating capacity of the muscle as the fascicles are more aligned with the line of pull (Degens et al., 2009b). Older muscles will also have a larger proportion of fat infiltration that contributes to the lower specific tension often observed in older than younger muscles (Degens and McPhee, 2013, Delmonico et al., 2009a, Goodpaster et al., 2001, Power et al., 2014).

Specific force reduction

The fact that muscle maximal force declines more than muscle mass in old age has led to considerable interest in the concept of “muscle quality”, meaning the force generated per unit muscle mass. In whole muscle, this has been termed “specific force”. Various explanations have been proposed, including an increase in fat infiltration of the muscle as well as a reduction in specific force of single muscle fibres, known as the specific tension (Canepari et al., 2010, Larsson et al., 1997, D'Antona et al., 2007). There is evidence that a reduced specific tension is due to oxidative-

damage causing alterations to the myosin head (Lowe et al., 2004, Lowe et al., 2001) and an age-related decrease in the myosin concentration in muscle fibres (D'Antona et al., 2003). One type of post-translational modification may be myosin glycation that has been reported in muscles of older individuals and in diabetes patients (Syrovy and Hodny, 1992). Indeed, glycation of myosin results in a reduced ability of myosin to propel actin in an *in vitro* motility assay (Ramamurthy et al., 2001). It remains to be seen whether myosin glycation is indeed a factor that contributes to the age-related muscle slowing.

Neural regulation

Another potential cause of the age-related reduction in specific force is a lower ability to voluntary activate a muscle (Morse et al., 2004). In addition, some fibres may not be activated at all, as they may have become denervated due to motor neuron loss. In a rodent study, this explained an 11% reduction in specific tension in old compared to young rats (Urbanchek et al., 2001). The good news is that most denervated fibres will eventually be re-innervated by sprouting from axons from surviving motor neurons, but this process is incomplete, resulting in a decrease in motor units and an increase in remaining motor unit size (Piasecki et al., 2018b, Piasecki et al., 2016b).

Causes of muscle weakness

Part of the age-related decrease in muscle mass may be caused by the progressive decrease in levels of physical activity as humans age (Ingram, 2000, Degens and Alway, 2006). In fact, one of the most intriguing properties of skeletal muscle is its enormous plasticity, reflected by the ability to respond to altered functional demands (Larsson et al., 2019). For instance, muscle adapts to endurance training with increased fatigue resistance and aerobic capacity, while repeated overloading causing

increased muscle strength and size (Jones et al., 1989, Salmons and Henriksson, 1981). On the other hand, disuse leads to atrophy and weakness.

Older muscles may have a compromised ability to maintain muscle mass, with reduced myofibrillar protein synthesis rate leading to reduced quality and quantity of motor protein due to disuse in older age (Larsson et al., 2019). This is part of a the larger issue of anabolic resistance shown with ageing, occurring from a blunting of the response from anabolic stimuli, these being the ingestion of amino acids and contractile loading of skeletal muscle (Breen and Phillips, 2013). Though it has been reported that increasing the volume of exercise at either 40% or 75% of one repetition max is able to overcome anabolic resistance to some extent (Kumar et al., 2012), with it being consistently shown that older individuals do respond with hypertrophy to resistance exercise (Fiatarone et al., 1990, Harridge et al., 1998). Not only force, but also the maximal shortening velocity of a muscle may be influenced by physical activity levels, where a high level of activity has also been linked to higher shortening velocity and specific tension in single muscle fibres (D'Antona et al., 2007). Undoubtedly, reduced levels of physical activity do contribute to the apparent age-related loss of muscle mass and strength. However, it is not the whole story as even masters athletes exhibit similar percentage annual loss in performance in a wide range of athletic events (Ganse et al., 2018) and the fibre type shift is opposite to that seen in disuse (Degens and Alway, 2006).

Besides lower physical activity levels, other factors must contribute to muscle ageing leading to the reduction in muscle quality observed. One of those, the ongoing denervation/re-innervation process due to motor neuron loss, is already described. It is, however, unclear what causes this loss of motor neurons. Fat and fibrotic infiltration during ageing with ageing is thought to decrease muscle quality, through the

impairment of force generation (particularly the lateral transfer of force) (Ramaswamy et al., 2011). Muscle architecture in ageing is influenced by dysregulated muscle remodelling, with myogenic progenitor cells switching to a fibrotic outcome (Shefer et al., 2006), possibly signifying intramuscular fat and fibrotic deposits are due to changes in satellite cell signalling (Vertino et al., 2005). Low-grade systemic inflammation in old age may be such a factor and has been suggested to play an important role in the ageing-related muscle wasting (Degens, 2010). Some of these circulating factors seem indeed to be linked to muscle weakness such as interleukin 6 (IL-6) and tumour necrosis factor α (TNFα) (Visser et al., 2002). Satellite cells key in muscle regeneration may have reduced ability to self-renew during ageing (Shefer et al., 2006). Possibly leading to apoptosis or senescence, due to an increase in proliferation (Chakkalakal et al., 2012, Sousa-Victor et al., 2014). Contributing to impaired regeneration of muscle with ageing, alongside a possible contribution to neuromuscular degeneration, leading to decreases in muscle quality (Carlson et al., 2001, Liu et al., 2017). It is also important to consider changes in reactive oxygen species (ROS) and leading to alterations in antioxidant defence systems seen with ageing. Part of the loss of the regeneration capacity of satellite cells could be the increase in ROS seen in older subjects, leading to damage of cellular macromolecules, suggesting redox balance plays an important role in muscle ageing (Minet and Gaster, 2012). In skeletal muscle, the antioxidant defence system has been shown to be upregulated during ageing (Sullivan-Gunn and Lewandowski, 2013). However, there is no further increase in antioxidant enzyme activities normally elicited from muscle contraction in older muscle, leading to a potential increase in oxidative damage to muscle cells (Vasilaki et al., 2006). The increase in ROS and attenuated antioxidant defences is thought to contribute to mitochondrial dysfunction. The increase in ROS

lead to mutations in mitochondrial DNA, impairing the electron transport chain due to the production of dysfunctional components, which in turn leads to a further increase in ROS (Miquel et al., 1980). Exacerbating the damage to muscle cells in ageing, leading to muscle atrophy in humans (Bua et al., 2006). During ageing there is also a decrease in hormones, such as a reduction in insulin-like growth factor-I (IGF-I), that may lead to a reduced anabolic environment (Lamberts et al., 1997). In addition, the reduction in sex hormones have been linked with muscle weakness in both older men and women (Van Vliet et al., 2005, Phillips et al., 1993). These alterations in the profile of circulating hormones may well contribute to the anabolic resistance seen in older individuals (Rennie, 2009), with studies showing a need for an increased protein consumption for a positive effect on lean mass in undernourished older individuals (Park et al., 2018).

1.4 Quantification of Body Composition in Humans

In the past four decades there have been considerable advancements in the study of body composition (BC). It is now possible to measure body organs, tissues and cells in very high detail (Malina, 2007). BC allows for accurate assessment of organs, tissues and cellular compartments for clinical diagnosis and to identify where intervention may be needed (Lee and Gallagher, 2008). Different techniques of assessments are available and they each have their merits and their weaknesses. A five-level framework can describe whole body, tissue, molecular, and atomic aspects of the human body. Often BC is represented in terms of protein, lipids, carbohydrate (usually dismissed due to small amounts held), minerals and water. To estimate body mass from these, the following equation is used:

Body mass = water + protein + mineral + lipids (Malina, 2007)

Generally, a two-compartment model is the preferred method of partitioning body mass: fat mass (FM) and fat free mass (FFM). Some models separate FFM into muscle, bone mineral and connective tissue. Of these components FM, and muscle are the most readily influenced by diet and activity (Malina, 2007). Clinicians and researchers are interested in finding an optimal balance between FFM, FM and total body mass to achieve health in the general population. This information can help in the design of interventions to keep older individuals physically independent and more robust as they age. The age related alterations in BC, especially the loss of muscle mass, not only leads to muscle weakness as discussed above, but can also cause metabolic problems (Tzankoff and Norris, 1977). BC is also an indicator of nutritional status and provides information on possible instances of malnutrition. Therefore, the accurate tracking of body composition throughout the lifespan is imperative to help achieve better health outcomes within populations. This can only be achieved through the use of a consistent and accurate method of BC estimation.

Computer tomography (CT) and MRI offer the greatest level of detail when assessing BC, providing an estimate of muscle volume, through the analysis of muscle cross-sectional slices, which can then be used to construct the full volume of the muscle. CT and MRI have also been used to quantify subcutaneous and intramuscular fat infiltration. CT and MRI are considered the “gold standard” to estimate skeletal muscle size and body composition in populations including children (de Ridder et al., 1992), healthy adults (Ross et al., 1996) and the elderly (Baumgartner et al., 1992). However, the acquisition of data from MRI and CT scanning is an expensive and time-consuming process. DXA is a quicker technique, but provides a 2-dimensional estimate of lean body mass, fat mass and bone mineral content. In all three modalities aforementioned,

there could also be issues with scanning larger individuals and it may be difficult to define water and intramuscular lipids.

Dual-Energy X-Ray Absorptiometry

The principle of DXA is that X-rays are absorbed to a different extent by different tissues. To perform the measurement a low and high pseudomonoenergetic beam is produced by a high-speed fan beam scanner X-ray tube and the absorption of the X-ray measured (Andreoli et al., 2009). DXA was initially used for the assessment of bone mineral density and diagnosing osteoporosis and osteopenia. It has now become clear that it can also be used to collect whole or regional data on body composition and because of its relative ease it has become a popular modality. However, there are some issues with the use of DXA to assess body composition, as the algorithms may be valid for young people, but not directly transferred for older people, due e.g. to an age-related increase in connective tissue, intramuscular fat and subcutaneous tissue. DXA sees non-adipose components of fat tissue, connective tissue (Wang et al., 1996) and non-mineral components of bone (Heymsfield et al., 1990) as FFM. It must also be noted that DXA measurements assume that the hydration status of FFM is 73%, but this can vary from 67-85% dependent on hydration status (Pietrobelli et al., 1998). This bias is likely to be small as it has been reported that no significant effect was seen on total fat percentage when hydration ranged from 68% - 78% (Kelly et al., 1998).

DXA scans are much more accessible, easier to use and interpret, as well as having considerably lower costs than MRI scanners. However, DXA may overestimate fat and mineral content, is unsuitable to distinguish separate muscle groups and lacks therefore the ability to asses muscle quality (Shaw et al., 2007). There is also an exposure to radiation when conducting DXA (ranging from 0.04 – 0.86 mrem), dependent on the make and model of the scanner and the dimensions of the patient

(Lee and Gallagher, 2008). However, this dose is much lower than normal daily exposure (1.69 mrem).

The first studies looking into the validity of DXA used the measure of whole body potassium to correlate against DXA measurements, as whole body potassium indicates the lean mass of a person (Womersley et al., 1972). It was found that appendicular lean mass was strongly correlated to total body potassium (r=0.94) (Heymsfield et al., 1990), though DXA was found to overestimate muscle mass when compared to measures by CT, with this overestimation increasing with increasing muscle mass (Heymsfield et al., 1990). It has been suggested that the overestimate of lean mass by DXA is due to assumptions made for protein or other material in adipose tissue and bone, therefore skewing the measure of lean mass (Heymsfield et al., 1990, Loenneke et al., 2016, Abe et al., 2015). However, adjustments to account for fat infiltration, none-bone mineral content and connective tissue do no remove this bias (Heymsfield et al., 1990, Kent-Braun et al., 2000)

Magnetic Resonance Imaging

MRI is considered the ‘gold standard’ of body composition measures alongside CT. Through the use of multiple image slices a 3D model of volume can be estimated (Narici et al., 1992, Erskine et al., 2009), which can be used to measure bone (Woodhead et al., 2001), adipose tissue (Kullberg et al., 2009), skeletal muscle mass (Baumgartner et al., 1992, Narici et al., 1992) and connective tissue within the target area. MRI measurements are based on the high-water content, and hence proton content of body tissue. The magnetic field aligns the spin axis of the protons that is disturbed by radio frequency waves. Cessation of the wave leads to realignment of the proton spins, and the speed at which this realignment occurs is dependent on the position of the protons in the molecules. The receiver coils collect the radio frequency

waves emitted by the protons when spin orientation is manipulated. The differing frequencies received indicate the type of tissue: light grey being muscle, dark grey connective tissue and white adipose tissue.

MRI as a modality is extremely accurate and reproducible and this is one of its main strengths. It can track small changes in mass over time as well as the ability to discern the individual muscles in a larger muscle group. MRI also does not emit radiation like CT or DXA scanning procedures, therefore making it an ideal candidate for repeated procedures in individuals. However, MRI machines and maintenance costs are high, and the scanning process and assessment of images takes skill and time, while some larger individuals may not be able to be scanner, all factors that lead to limited availability of this equipment.

1.5 Mobility and Function measurements

Six-minute walk test

The assessment of functional capacity in terms of everyday living is essential for the diagnosis of functional limitation in the elderly and especially the frail elderly. While this can be done through self-reported measures, they carry the risk of over and under-reporting. Therefore, more objective measures should be used, such as a shuttle walk test or 12-minute walk test. More commonly used is the 6-minute walk test (6MWT) (Enright, 2003) that is an inexpensive, valid measurement, with good levels of reproducibility (Pollentier et al., 2010, Costa et al., 2018). The 6MWT has also been reported to correlate with an individual's peak oxygen uptake and is widely used to measure the response to therapeutic interventions (Enright, 2003).

Timed Up-and-Go

The timed-up-and-go (TUG) test is a useful functional measure because it includes rising from a seated position, a short walk and negotiating a turn. Originally a 'Get-Up and Go' Test was developed (Mathias et al., 1986) to assess these factors of functional mobility, which was modified to enable an integrated measure of balance, gait speed and functional capacity (Podsiadlo and Richardson, 1991). This test has been found to be reproducible over time and between assessors, alongside fulfilling the criteria of a functional measurement (Solomon, 1988).

Balance

Nearly every neuromuscular disorder results in degeneration of balance control, with this loss of balance comes an increase in the chance of falls and in the elderly this often leads to death (Winter et al., 1990). It is important that individual's neural control is able to handle many of the perturbations that occur during phases of locomotion and therefore improve fall outcomes in older individuals (Winter et al., 1990). Thus, the measurement of balance allows further assessment in the neuromuscular changes that occur during the ageing process.

Muscle power

The assessment of muscle power is most commonly measured through the counter movement jump, which can feedback data such as jump height, peak power, and peak velocity. Therefore, helping determine an individual's neuromuscular status, the use of counter movement jump without arm swing has be shown to be a reliable performance measure when average values are taken (Claudino et al., 2017). Though it does appear that jumping mechanics do change with ageing, partly due to a

simultaneous coordination of muscle activation with advanced age (Haguenauer et al., 2005).

Isometric maximal voluntary contraction torque

Isometric strength tests are those where a force is produced with the lengths of muscle remaining relatively fixed. Lower limbs are usually assessed by asking an individual to push or pull against bar or cuff attached to a strain gauge. The use of such measures enables the assessment of maximal voluntary activation, giving insights into changes of maximal voluntary activation in such target populations (i.e. sarcopenic) (Bergamin et al., 2017, Nuzzo et al., 2018). It is important that such functional tests use best practice and are repeatable and reliable, isometric strength tests tend to be free of systematic bias and a have good test-retest reliability in healthy older adults (Bergamin et al., 2017, Nuzzo et al., 2018). The method used within this thesis to test contractile properties of human quadriceps longitudinally was shown to have excellent reproducibility (Appendix 1).

Voluntary activation

The use of electrical stimulation to assess human muscle contractile properties has been well established (Gerrits et al., 2001, Chan et al., 1999, Hunter et al., 1999, Degens et al., 2005, Morse et al., 2007b, Wust et al., 2008). To assess the voluntary activation an electrical evoked twitch is superimposed on the maximal voluntary contraction and the amount of extra force, when compared to an electrically-evoked twitch preceding the voluntary contraction, gives a measure of the degree of voluntary activation.

Besides the assessment of voluntary activation, electrical stimulation allows the determination of muscle contractile indices such as the force frequency relationship,

fatigue resistance, maximum rate of contraction and relaxation while avoiding possible motivational bias. The contractile properties determined in this way appear to correlate with a variety of molecular or histochemical muscle features (Harridge et al., 1998).

When performing repeated measures, it is important to know how reproducible the measurements are. This is particularly important when one wants to know the impact of an intervention on the contractile properties of the muscle, especially when one considers how muscle adapts to changes in functional demands (Baar and Hargreaves, 2011, Koopman and van Loon, 2009, Nuzzo et al., 2018).

1.6 Longitudinal observations

Large cross sectional studies which have been well-designed through the use of multiple methods of imaging, functional and questionnaire data, do provide sound measure of variability between young and old, that highlight a general downward trend in muscle mass (Mitchell et al., 2012). However, there is always the risk that systematic influences may skew the results, such as intergenerational differences for example, those born during rationing. Longitudinal studies overcome such systematic issues and are able to track estimates of muscle mass in the same population over a time, giving a more accurate and robust model of ageing. However, the current literature describing muscle mass changes longitudinally is limited, due to the inherent difficulty of collecting data over extended timescales.

Frontera et al. (2000a) conducted a 12-year follow up study, using computed tomography, which alongside MRI is seen as the gold standard in body composition measurements. Thigh cross sectional area was calculated and then separated into anterior and posterior thigh muscles and the changes were reported for a small cohort (n=7, 65.4 ± 4.2 yr) of healthy sedentary men transitioning from their seventh decade

to eighth. A 1% annual decline in thigh CSA was reported, which outlined the accelerated loss of mass postulated to occur during this period, describing a larger loss than the estimated 0.5% annual decline reported by Maden-Wilkinson et al. (2014) in a cross sectional study of the same muscle group between young (early twenties) and old (early seventies) independent living adults. No differential rate of loss between extensor and flexor muscles was found, whereas an increased loss of extensor muscles compared to flexor muscle between young and old was reported by Maden-Wilkinson et al. (2014).

Conversely, Hughes et al. (2002) reported losses of 0.2% in a larger sample size, describing individuals through their seventh decade, which is markedly lower than that reported by Frontera et al. (2000a) and even lower than reported in a cross-sectional study (Maden-Wilkinson et al., 2014). Similar losses were then reported by Dey et al. (2009) in an older cohort (75-80yrs) with an annual loss of 0.18%. Both of these studies reported changes in fat free mass, using hydro-densiometry and bioelectrical impedance to estimate the muscle changes. These methods may lack the sensitivity and specificity required to track changes in the muscle during ageing and therefore should be interpreted with caution.

Larger-scale work as part of the Health, Ageing and Body Composition study was conducted. This work described the changes in much larger sample sizes, with Delmonico et al. (2009a) describing changes over a 5-year period using computed tomography and Koster et al. (2011) using DXA over a 7-year follow up in well-functioning (reported no physical limitations and were not currently undergoing treatment for cancer) men and women during their eighth decade. The findings from Delmonico et al. (2009a) reported 0.98% annual thigh losses in men which is similar to the values published by Frontera et al. (2000a) which also used computed

tomography to assess muscle mass. However, Koster et al. (2011) found a lower annual loss of muscle mass over 7-years in men (0.8%), this may be due to differences in methodology. For example, previous work suggests an overestimation of muscle mass with the use of DXA (Maden-Wilkinson et al., 2013b, Visser et al., 1999, Levine et al., 2000, Shih et al., 2000). Men suffered from larger age-related loss of muscle mass than women in both the Health, Ageing and Body Composition reports (Koster et al., 2011, Delmonico et al., 2009a), with women reporting an annual decline of 0.64% (Delmonico et al., 2009a) and 0.7% (Koster et al., 2011). Suggesting there is disparity between how men and women age which has also been suggested in other studies (Iannuzzi-Sucich et al., 2002, Castillo et al., 2003, Janssen et al., 2000c).

Of the longitudinal studies conducted currently, both Koster et al. (2011) and Delmonico et al. (2009a) represent the most reliable data in free-living older adults, though these still lack the detail to fully characterise age-related loss in muscle mass.

1.7 Summary of current research

The majority of our knowledge of effects of old age on musculoskeletal structure and function come from cross-sectional studies that compared data from young with data from older adults to try to understand mechanisms behind the decrease in muscle mass with ageing and the larger proportional loss of function. However, there is always the potential bias related to differences in genotype and changes in life-style over the decades that human ageing takes that may cause unintended bias in cross-sectional studies. Such bias can be overcome in longitudinal studies of changes in muscle size and function with ageing. Such studies are the minority, often only covering relatively simple assessments that can be carried out easily and cost effectively as part of large epidemiological studies or health assessments. They have not had an integrated

approach of structural and functional changes in skeletal muscle and their impact on the ability to perform activities of daily life. Which can only be achieved with multiple validated testing procedures on a cohort. In addition, many studies investigating the impact of ageing on muscle mass have used DXA with little consideration of the possible over- or underestimation of muscle mass by this technique. It is therefore key that these factors are uncovered and described in detail to give a clearer view of ageing into the eighth decade, especially as humans are now living longer though towards the end of their lifespan not necessarily in good health. The maintenance of mobility and health into old age a key current challenge, with the development of mobility impairments of leading to a decrease in quality of life. Consequently bringing to the forefront and encouraging the implementation of interventions that can attenuate such changes.

1.7 Unresolved issues

There are still a number of unresolved issues when it comes to muscle ageing:

- Whether DXA measurements are appropriate and accurate to track changes in muscle mass longitudinally in older adults.
- What the underlying causes of the increased proportional loss of force is ageing
- Whether there is an accelerated decline in muscle mass seen in healthy free living septuagenarians during later ageing, compared to the linear decline described in cross sectional studies
- How functional capacity in healthy individuals entering their 8^{th} decade alters, in comparison to relative changes seen over prior decades

1.8 Aim of the Thesis

The overall aim of the work in this thesis was to track and characterise longitudinal changes in muscle size and function and how this impacts on the ability to perform activities of daily life over a 5-year period, in healthy free-living septuagenarians. Which is of particular interest and importance due to the increasing proportion of the population over 60 years of age. It is estimated this population will more than triple within 50 years to over 2 billion by 2050 (WHO, 2018). The participants in this work were part of a larger European-wide cross-sectional study (MYO AGE) to describe the aforementioned characteristics. The objectives of the work presented in this thesis were:

1) To assess the reliability of DXA measurements against MRI to assess longitudinal changes in muscle mass in older populations.

2) To uncover the gross functional basis of the age-related changes in mobility seen in older individuals.

3) To describe the influence of fibre atrophy, fibre loss, in situ specific force, and voluntary activation to muscle weakness seen with ageing.

The following work presents novel data to address each of the above objectives.

This work was proceeded by a larger project known as “Myoage” (nr: 223576), funded from the European Commission, alongside the Medical Research Council as part of the Life Long Health and Wellbeing initiative (MR/K/025252/1) and is entirely the work of James Cameron.

Chapter 2

Five-year longitudinal changes in thigh muscle mass of septuagenarian men and women assessed with both DXA and MRI

2.1 Abstract

Magnetic resonance imaging (MRI) and dual-energy absorptiometry (DXA) were used to assess changes in thigh lean mass in septuagenarian men and women during a 5-year longitudinal study. Twenty-four older individuals participated in the study (10 men; 71.6±4.1 y: 14 women; 71.3±3.2 y at baseline). Thigh MRI and whole-body DXA scans were used to estimate changes in thigh lean mass. Both MRI and DXA showed that thigh lean mass was reduced by approximately 5% over the 5-year period in both men and women (P=0.001).The percentage loss of muscle mass determined with MRI and DXA showed moderate correlation (R^2=0.466; p<0.001). However, DXA over-estimated thigh lean mass at both baseline and follow-up by 0.86 and 0.82 kg, respectively. Bland Altman analysis showed that the average atrophy over five years of follow-up measured by DXA was only 0.18% greater than MRI, where the limits of agreement between DXA and MRI were ±10.4%. Baseline thigh lean mass did not predict the percentage loss of thigh lean mass over the 5-year period (R^2=0.003; P=0.397), but a higher baseline body fat percentage was associated with a larger loss of thigh muscle mass in men (R^2=0.677; P<0.003) but not women (R^2=0.073; P<0.176). In conclusion, 1) DXA and MRI showed a similar percentage loss of muscle mass over a 5-year period in septuagenarian men and women that 2) was independent of baseline muscle mass, but 3) increased with higher baseline body fat content in men.

2.2 Introduction

Ageing is accompanied by changes in muscle mass that are thought to contribute to reduced physical function and vigour, and the eventual loss of independence in old age (Doherty, 2003a). This loss of muscle mass and physical function has been described as sarcopenia (Rosenberg, 1989b). By the 8th decade, muscle mass has declined by around 30% from peak values, with these losses principally coming from the atrophy of type II fibres (Barnouin et al., 2017b) and loss of muscle fibres (McPhee et al., 2018). The loss of myofibers seen in ageing is thought to be a consequence of motor neuron death and it has been reported that up to 50% of motor units are lost by the 8th decade (Piasecki et al., 2018a). It should also be noted that there might be a differential rate of age-related loss of muscle mass between men and women, with men thought to suffer to a greater degree (Iannuzzi-Sucich et al., 2002, Castillo et al., 2003, Janssen et al., 2000c). With increased adipose tissue also thought play a role in the rate of age-related loss of muscle mass (Newman et al., 2003).

The major problem with most studies of human ageing is that they are cross-sectional and it is important to develop and validate methods to assess changes in muscle bulk in longitudinal studies. Muscle imaging techniques allow the non-invasive evaluation of skeletal muscle size and architecture (Lee et al., 2013) and include bio-electrical impedance (BIA), CT, DXA and MRI (Heymsfield et al., 1990, Narici et al., 1992, Wang et al., 1996, Visser et al., 1999). CT and MRI are generally considered the gold standard, allowing the accurate assessment of muscle cross-sectional area, muscle mass and intramuscular adipose tissue content. Nonetheless, these techniques are expensive and consequently DXA (Baumgartner et al., 1998) and BIA (Janssen et al., 2000a) are frequently used to identify sarcopenia.

Previously, good correlations have been found between muscle size estimations with CT, DXA and MRI in similar groups of individuals (Fuller et al., 1999, Visser et al., 1999, Levine et al., 2000, Segal et al., 2009, Maden-Wilkinson et al., 2013b). However, despite a strong correlation (R^2=0.90 young, R^2=0.83 old) in a large cross-sectional cohort study, thigh muscle mass was over-estimated by DXA as the slope of the DXA-MRI relationship was steeper than 1 and had an intercept of approximately 0.4 kg (Maden-Wilkinson et al., 2013b). In addition, DXA underestimated the percentage difference in muscle mass between young-adults and older people (Maden-Wilkinson et al., 2013b), suggesting that DXA underestimates the age-related loss of muscle mass. It remains to be seen, however, whether longitudinal changes in skeletal muscle mass of older people can indeed be determined with DXA, or that this method also underestimates the loss of muscle mass beyond the age of 70 as a consequence of the increased fraction of the intercept of the whole signal.

Therefore, the purpose of the present study was to compare changes in muscle bulk as measured by DXA and MRI in a 5-year longitudinal study of men and women in their 8th decade. It was hypothesised that DXA underestimates the loss of muscle mass when compared with MRI. In addition, we studied whether the rate of muscle loss is 1) negatively related to baseline muscle mass and/or 2) positively related to baseline body fatness.

2.3 Methods

Participants and ethical approval

The participants are a subgroup from the cross-sectional MYOAGE study (www.myoage.eu) (McPhee et al., 2013). The participants were recruited from the local community (Manchester, UK) and were asked 5 years later to return for a follow-up study. Data presented in this report are from the twenty-four older participants that returned (10 men, 14 women), with a drop-out rate of 74% from the original cohort of 66 men and women. Written informed consent was obtained from each participant before partaking in both the first and the follow-up study. The studies conformed to the Declaration of Helsinki and were approved by the local ethics committee of the Manchester Metropolitan University. Participant characteristics are presented in Table 2.1. All individuals were community dwelling, socially active and classed as healthy. Exclusion criteria were: known musculoskeletal or cardiovascular diseases, any limb fractures within 5 years of testing, hip or knee replacement in the previous 2 years, immobilised for greater than 1 week 3 months prior to testing, institutionalisation, unable to walk 250 m unassisted, chronic pain syndrome, metabolic disease, chronic obstructive pulmonary disease, or neurological disorders (e.g. Parkinson's).

Anthropometry

While wearing light indoor clothing, body mass was recorded on a digital scale to the nearest 0.1 kg. Standing height was measured using a stadiometer to the nearest 1 mm. Body mass index (BMI) was calculated as body mass (kg)/(height (m)^2).

Duel Energy X-ray absorptiometry

Participants lay supine on the scanning bed wearing a medical gown. A total body DXA (Lunar Prodigy Advance, GE Healthcare, Chicago, USA) scan was performed to

measure total body composition and bone mineral density. Estimations of total lean mass and fat mass were obtained using Prodigy, Encore 2006 v10.50.086 software (GE Healthcare). To estimate the fat mass, bone mineral content and lean mass in the thigh of the dominant leg, the thigh was demarcated by one border proximally and parallel to the greater trochanter and another through the knee joint line, as described previously (Segal et al., 2009, Maden-Wilkinson et al., 2013b) (Fig. 2.1A). All DXA analyses were completed by the same investigator. Each standard total body scan took 295 s with an estimated skin entrance dose of 0.4 μGy (GE Healthcare, Lunar encore, Safety and Specification Manual). Typically, the estimates of lean mass by DXA software packages include connective tissue, non-mineral components of bone and non-adipose components of fat tissue alongside muscle mass. As the contribution of these factors is unclear and possible changes of these components with aging unknown, we did not correct for these potential confounders. The system was calibrated with the same whole-body phantom at baseline and at 5 years follow up before each scan.

Magnetic Resonance Imaging

In six of the participants, thigh volume was measured using a 0.25-T MRI scanner (G-Scan, Esaote, Genova, Italy). The participant was in a supine position in the scanner and multiple 3.1 mm thick serial transverse sections were acquired every 25 mm from the proximal to the distal heads of the femur of the dominant leg using a turbo 3D T1-weighted protocol (matrix 256 x 256, TR 40 ms, TE 16 ms). The cross-sectional area of the four quadriceps muscles and other thigh muscles (hamstrings, abductors and adductors) in each slice (Fig. 2.1B) were determined using computer imaging software (OsiriX medical imaging software, OsiriX, Atlanta, USA). Visible fat and connective tissue were not included in the measurement region, with all muscles measured three

times with the average recorded. Estimated total thigh volume was calculated through the summation of the cross-sectional area of each head of the individual quadriceps muscles and other muscles in each slice, multiplied by the distance between slices. It was not possible to measure the rectus femoris (RF) beyond the head of the femur, due to limitations of coil size. Therefore RF volume for the proximal 10% was calculated assuming conical volume from the last transverse section to the origin of the muscle, identified using ultrasound (Esformes et al., 2002). MRI volumes were converted to mass by multiplying by the density of muscle tissue 1.04 g.cm-3 (Snyder et al., 1975), aiding the comparison between measures. It has previously been shown that thigh muscle volume can be calculated from a single scan (Morse et al., 2007a, McPhee et al., 2009). In a subset of six subjects, a good correlation was found between the measured and the calculated thigh muscle volume ($R^2 = 0.89$; $p=0.007$) also in this cohort. Consequently, in the remaining 18 participants, thigh volume was estimated from a single scan taken at 60% of the length from distal-to-proximal femur (Morse et al., 2007a).

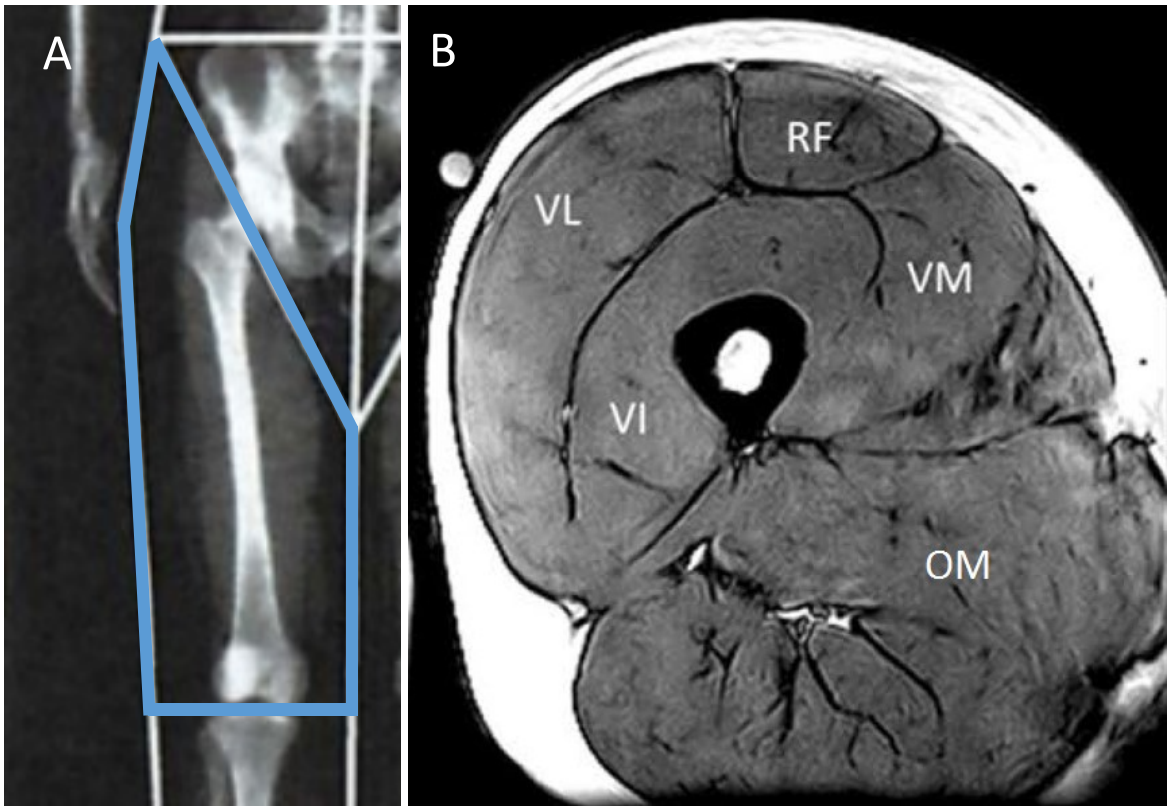

Figure 2.1. **(A)** Example of dual energy X-ray absorptiometry (DXA) image showing regions of interest of the thigh. **(B)** Magnetic resonance imaging (MRI) image of the thigh muscles. VI: vastus intermedius; VL: vastus lateralis; VM: vastus medialis; RF: rectus femoris; OM: other muscles.

Statistics

Data were analysed using SPSS v22 (IBM, 2015). Repeated-measures ANOVA with "within subject factor" time (baseline and follow-up) and "between subject factor" gender was used. A gender*time interaction indicated that men and women changed differently over time. Linear regression analysis was conducted to consider correlations between measurements. Statistical significance was accepted as $p<0.05$. Data were expressed as mean ± standard deviation unless stated otherwise. Bland Altman analysis (Bland and Altman, 1999) was used to determine the limits of agreement between DXA and MRI. The test-retest variability was given as the

coefficient of variation (CVp), which was calculated as the SD of the differences between MRI and DXA as a proportion of the mean: $CVp = \sqrt{((\sum CV_i^2)/n)}$.

2.4 Results

Participant Characteristics

Women were shorter, had lower FFM, appendicular lean mass (ALM), and bone mineral density (BMD) than men (Table 2.1; $p<0.001$). The participants lost about 1 cm in stature over the 5-year period as well as having lower FFM and ALM ($p\leq0.001$), irrespective of gender. The gender*time interactions for body mass ($p=0.029$) and BMI ($p=0.019$) were reflected by a decrease in body mass and BMI in women, but not in men. There was no significant change in Fat mass and BMD over the 5-year period.

Table 2.1. *Participant characteristics*

	Women (n=14)			Men (n=10)			Significant differences		
	Baseline	Follow-up	*% change*	Baseline	Follow-up	*% change*	Time	Gender	Gender*Time
Age (years)	71.3±3.2	76.2±3.3		71.6±4.1	76.2±4.4		P=0.000	P=0.923	P=0.193
Body mass (kg)	65.5±10.4	63.4±10.9	-3.5	83.6±15.2	83.9±15.1	0.5	P=0.079	P=0.000	P=0.029
Height (m)	1.61±0.07	1.60±0.06	-0.5	1.74±0.08	1.73±0.08	-0.5	P=0.000	P=0.000	P=0.874
BMI (kg/m²)	25.6±5.47	25.0±5.5	-2.0	27.7±4.4	28.1±4.1	1.5	P=0.798	P=0.123	P=0.019
FFM (kg)	39.0±3.1	37.7±3.1	-3.5	55.3±8.1	54.5±7.5	-1.5	P=0.001	P=0.000	P=0.378
FM (kg)	24.1±9.4	23.5±10.3	2.5	25.0±10.4	26.1±10.3	4.5	P=0.580	P=0.604	P=0.075
FM (%)	37.0±9.2	36.9±9.94	0	30.2±9.2	31.5±8.7	4.5	P=0.236	P=0.056	P=0.172
ALM (kg)	17.4±1.8	16.7±1.75	-4.0	25.7±4.0	24.6±3.7	-5	P=0.000	P=0.000	P=0.206
BMD (g/mm²)	1.07±0.10	1.07±0.10	0	1.25±0.12	1.26±0.12	1.0	P=0.352	P=0.000	P=0.519

Data shown as mean ± SD. *Abbreviations*: BMI: Body Mass Index; FFM: Fat Free Mass; FM: Fat Mass; ALM: Appendicular Lean Mass; BMD: Bone Mineral Density.

Correlations between MRI and DXA

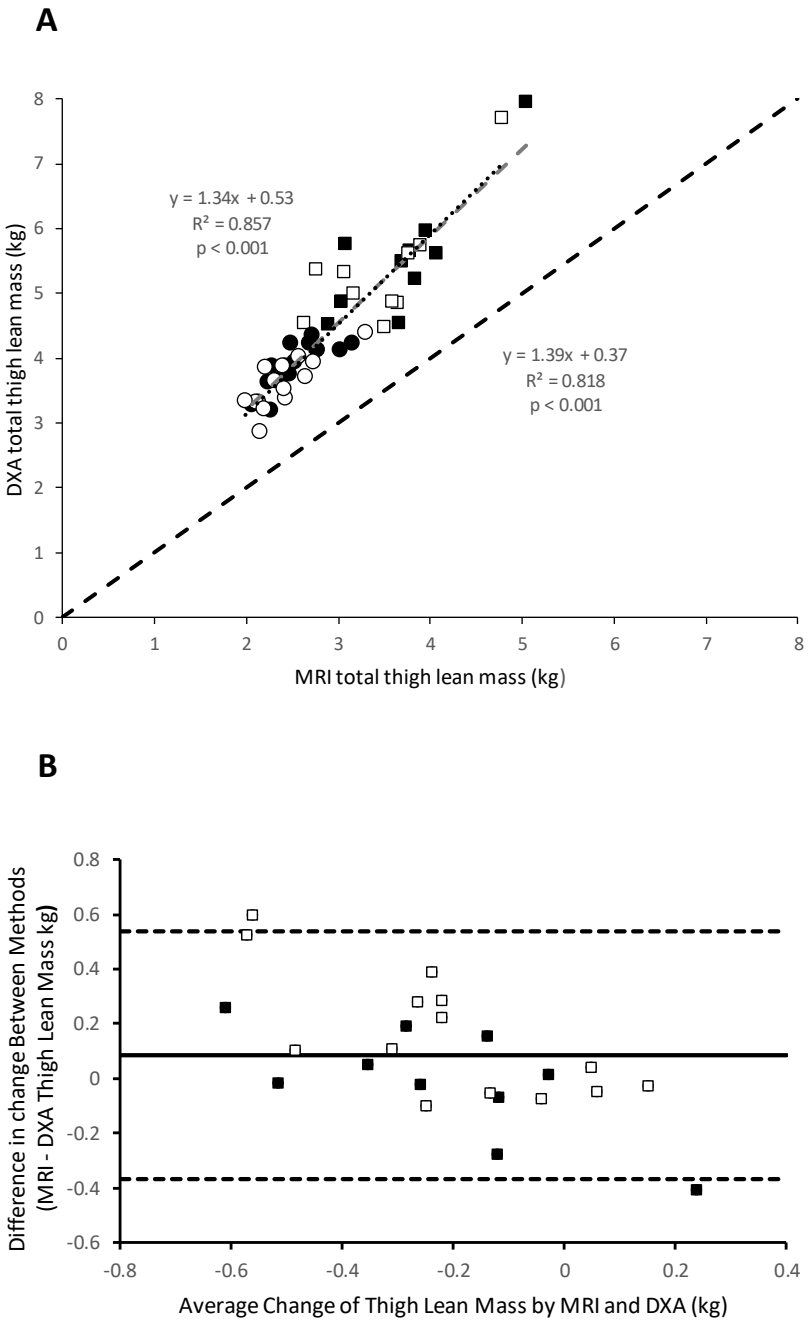

***Figure 2.2. (*A**)** The relationship between thigh lean mass as determined by DXA vs as determined by MRI. ■: men and ●: women at baseline, and □: men and ○: women at follow-up. ---: line of identity; ---: regression line at baseline; ⋯: regression line at follow-up. Equations – left: baseline; right: follow-up. **(B)** Bland-Altman plot to show the absolute agreement between MRI and DXA; ■: men and □: women. Horizontal dashed lines represent 1.96 standard deviation above and below the average difference between methods, depicting levels of agreement (+0.54 kg upper level of agreement and -0.37 kg lower level of agreement). Solid horizontal line represents the bias between methods (DXA shows a 0.09 kg larger loss of muscle mass than MRI over the 5-year period).

Both MRI and DXA showed that men had larger muscles than women (Table 2.2; $p \leq 0.001$). Figure 2.2A shows the correlation between thigh muscle size as measured by DXA and MRI for values at baseline ($R^2=0.857$; $p<0.001$) and at the 5-year follow-up (follow-up $R^2=0.818$; $p<0.001$). The regression lines of the two correlations were very similar.

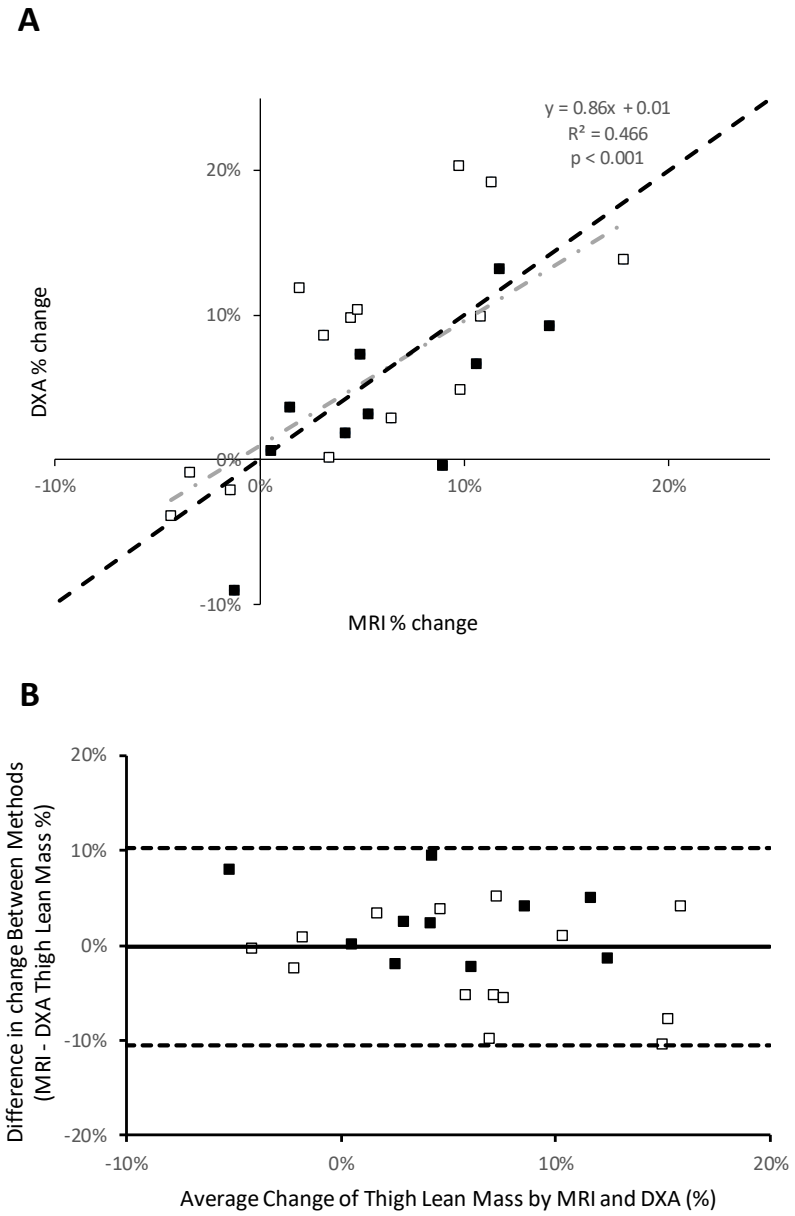

Figure 2.3. **(A)** The relationship in men and women between thigh lean mass percentage change as determined by DXA vs MRI. ■: men and □: women; ---: line of identity; -·-: regression. **(B)** Bland-Altman plot to show the percentage agreement between MRI and DXA. ■: men and □: women. Horizontal dashed lines represent 1.96 standard deviation above and below the average difference between methods, depicting levels of agreement (+10.2% upper level of agreement and -10.6% lower level of agreement). Solid horizontal line signifies the 0.18% larger decrease in muscle size determined by DXA than by MRI.

When analysing data showing the changes to muscle size over the 5-year follow-up, Bland Altman plots (Fig. 2.2B) showed a 0.09 kg larger loss measured by DXA compared with that measured by MRI. Limits of agreement between DXA and MRI was ±0.453 kg. The percentage loss of muscle mass determined with MRI and DXA showed moderate correlation (R^2=0.466; p<0.001; Fig. 2.3A). Bland Altman plots (Fig. 2.3B) show a 0.18% lower muscle loss measured by MRI compared with DXA and the limits of agreement between DXA and MRI was ±10.4%. The overall pooled co-efficient of variation (pCV) between MRI and DXA over 5 years was 0.045%.

Longitudinal changes in thigh lean mass

Table 2.2. *Measurements of thigh muscle size by dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI).*

	Women (n=14)			Men (n=10)			Significant differences		
	Baseline	Follow-up	*% change*	Baseline	Follow-up	*% change*	Time	Gender	Gender*Time
MRI Quadriceps muscle lean mass (kg)	1.05±0.16	1.01±0.16	-4.5	1.63±0.31	1.48±0.28	-8.8	P=0.001	P=0.001	P=0.61
MRI Other muscle lean mass (kg)	1.39±0.15	1.30±0.18	-6.4	1.94±0.34	1.86±0.35	-3.8	P=0.001	P=0.001	P=0.756
Quadriceps:other muscle ratio	0.76±0.08	0.78±0.11	0.00	0.85±0.15	0.81±0.13	-0.05	P=0.517	P=0.224	P=0.078
MRI Total thigh lean mass (kg)	2.44±0.29	2.31±0.31	-5.5	3.56±0.57	3.35±0.57	-6.1	P=0.001	P=0.001	P=0.228
DXA Thigh lean mass (kg)	3.89±0.36	3.59±0.40	-8.0	5.55±0.98	5.34±0.93	-4.0	P=0.001	P=0.001	P=0.529
MRI:DXA (ratio)	0.65±0.07	0.67±0.06	2.48	0.67±0.07	0.66±0.09	-2.32	P=0.967	P=0.904	P=0.039

Data shown as mean ± SD.

MRI showed a similar percentage decrease in thigh muscle size in men and women (Table 2.2). The percentage rate of loss of thigh lean mass did not differ significantly between DXA and MRI (p=0.841), as indicated by similar MRI:DXA ratios (Table 2.2) for thigh muscle size at baseline and 5-year follow up (p=0.967).

It is possible to distinguish the quadriceps and other muscles in the thigh with MRI to investigate possible differential atrophy between thigh muscles. The ratio of quadriceps to other muscles was similar in both genders at baseline (Table 2.2; p=0.224) and the absence of a significant age-related change in this ratio (Table 2.2; p=0.517) indicated that the atrophy was similar in both muscle compartments.

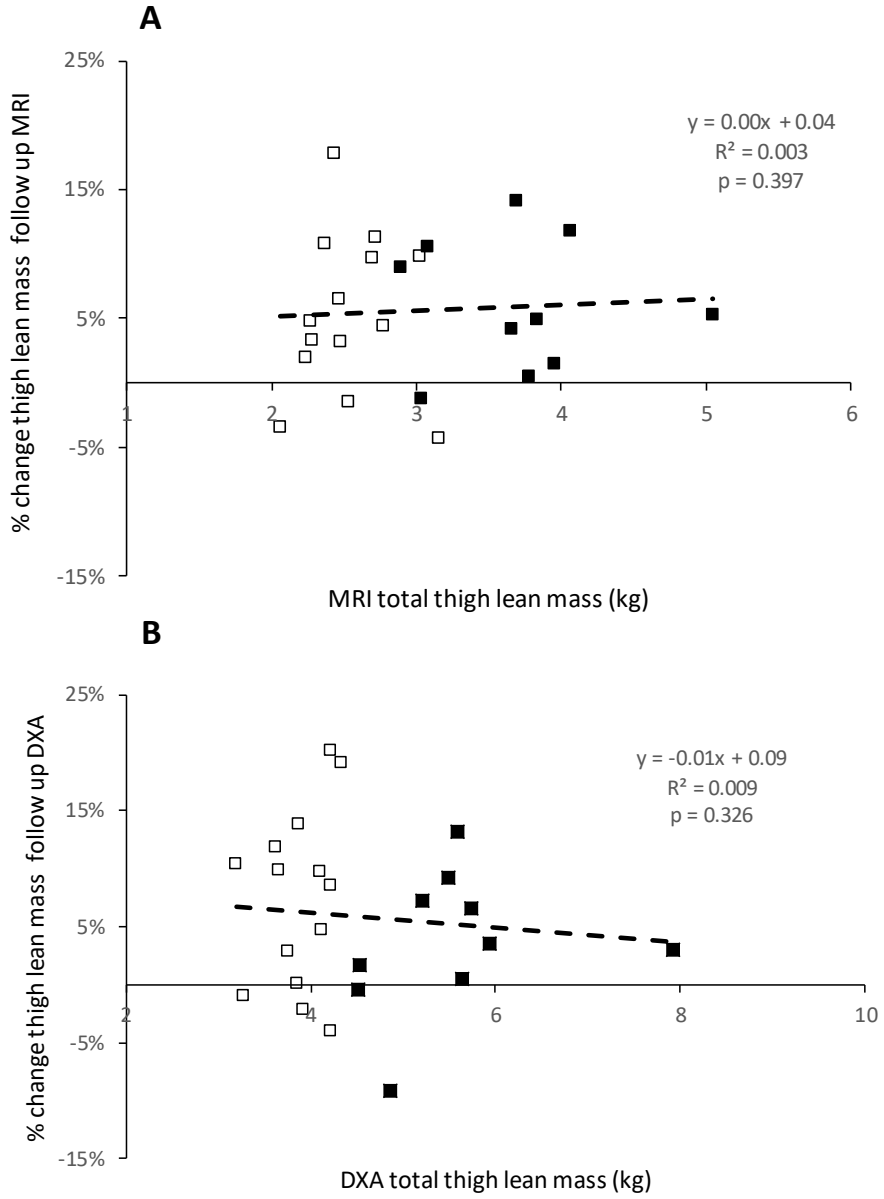

Figure 2.4. **(A)** The association between baseline thigh muscle volume and % change of thigh muscle volume at follow up in magnetic resonance imaging (MRI) **(B)** Association between baseline thigh muscle volume and % change of thigh muscle volume at follow up in dual energy X-ray absorptiometry (DXA). ■: men and □: women; ---: regression line.

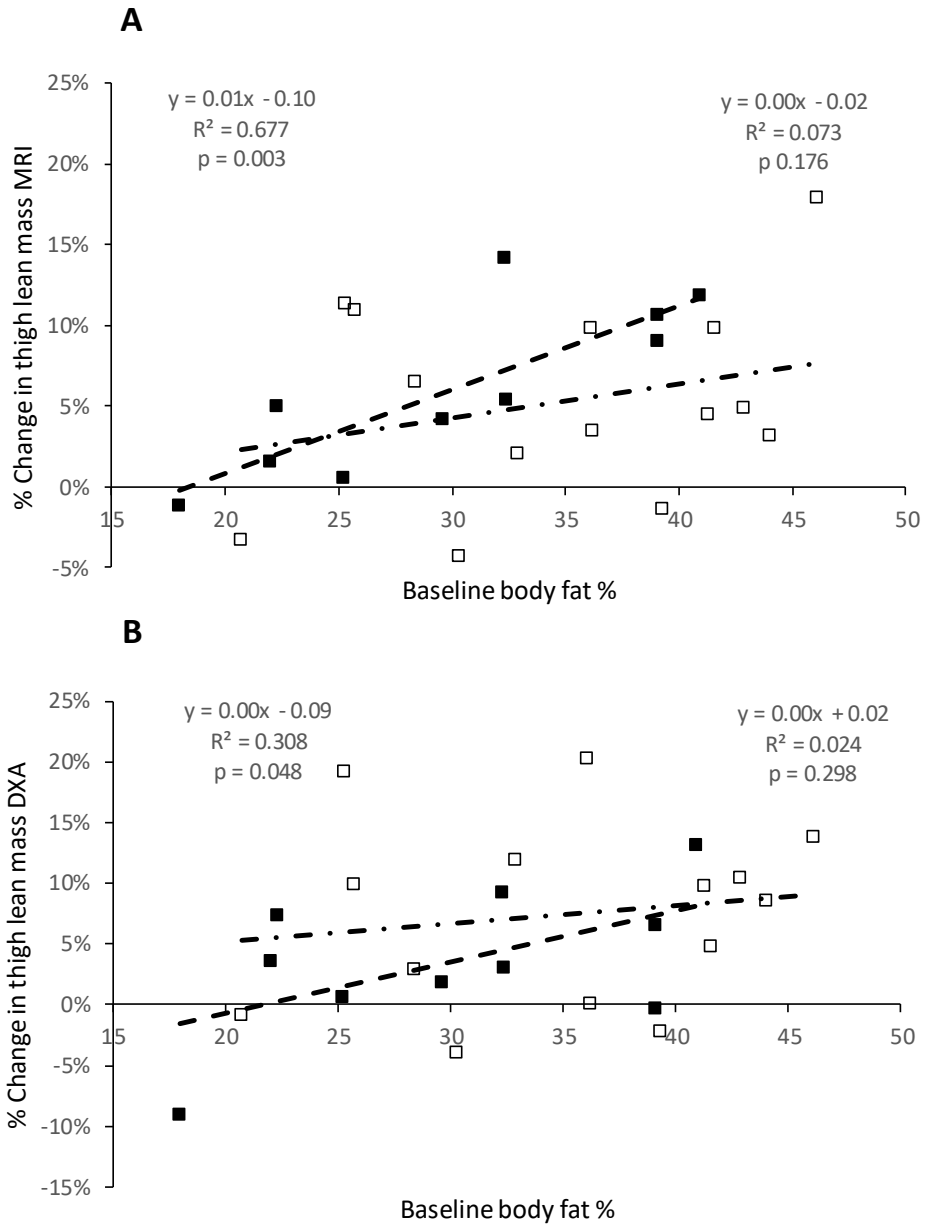

Figure 2.5. **(A)** Baseline body fat % association with the percentage change in muscle volume on follow up, as measured by magnetic resonance imaging (MRI) and ***(B)*** dual energy X-ray absorptiometry (DXA). ■: men and □: women; -- regression line men; -·- regression line women; Equations – left: men; right: women.

There was no significant correlation between baseline thigh muscle volume and the percentage decline over the 5-year period in either men or women when measured by either MRI (Fig. 2.4A; R^2=0.003; p=0.397) or DXA (Fig. 2.4B; DXA: R^2=0.009; p=0.326). However, baseline body fat percentage was associated with a larger percentage decrease in muscle volume following the 5-year period in men (Fig 2.5A MRI: R^2=0.677; p=0.003; Fig 2.5B DXA: R^2=0.308; p=0.048), but not in women (Fig 2.5A MRI: R^2=0.073; p=0.176; Fig 2.5B DXA: R^2=0.024; p=0.298).

2.5 Discussion

It has previously shown that DXA provides higher values for thigh muscle mass than MRI measurements and that, when comparing young and old, MRI measurements suggest a greater age-related decline in muscle mass than that obtained from DXA measurements (Maden-Wilkinson et al., 2013b). DXA is a convenient method to assess body composition and muscle mass but our previous cross-sectional observations raised concerns about its suitability for use in longitudinal studies of changes in muscle mass and progression of sarcopenia. In the present study it was confirmed that DXA gives higher values for thigh muscle mass than MRI and this was extended to show that the percentage loss of muscle mass over a 5-year period was similar for DXA and MRI. We also showed that the percentage loss of muscle mass in the 5-year period was similar in 1) quadriceps and hamstring muscles, 2) recreationally active healthy older men and women, 3) was independent of baseline muscle mass, 4) was greater in men with a higher baseline body fat percentage, and 5) that the rate of muscle decline was higher than that estimated from cross-sectional comparisons of people *aged in their 20s compared with those in their 70s.*

DXA vs. MRI

MRI total volume measurements are widely regarded as the gold standard though these measurements are both time consuming and costly. The cost and time can be reduced significantly by calculating muscle volume from a single MRI scan. Here we show that this is not only possible in young men (Morse et al., 2007a) and older men and women (Maden-Wilkinson et al., 2014), but that even the percentage decrease in thigh muscle cross-sectional area over a 5-year period correlated strongly with the percentage decrease in thigh muscle volume ($y=1.03x–0.04$; $R^2=0.875$; $p=0.003$). This indicates that estimating changes in muscle size from single MRI transverse sections taken at 60% of femur length is sufficient to assess changes in muscle volume longitudinally.

While the use of a single MRI scan already saves considerable time, and hence cost, MRI and CT are not commonly available. DXA has become a popular modality to assess body composition and muscle mass in large cohort studies, due to its wider availability and ease of use (Ellis, 2000, Visser et al., 2003, Goodpaster et al., 2006, Zhong et al., 2012, Santanasto et al., 2017). Although a good correlation was found between the thigh muscle mass determined by DXA and MRI in both men and women at both baseline and follow-up, DXA consistently overestimated the muscle mass due to a positive intercept and a slope of the regression line greater than 1. Such a positive intercept has been seen before in young adults and older people (Visser et al., 1999, Levine et al., 2000, Shih et al., 2000). This is also in line with previous work shown in a large cohort study reporting a positive intercept and a slope steeper than 1 (Maden-Wilkinson et al., 2013b). It has been suggested that protein or other material in adipose tissue may contribute to this over estimation of muscle mass by DXA (Abe et al., 2015, Loenneke et al., 2016). However, in our previous study, adjustments to account for

connective tissue, fat infiltration and none-bone mineral content of bones (Heymsfield et al., 1990, Kent-Braun et al., 2000) did not remove this bias.

In practice, and when following changes over a relatively short time scale of 5 years, the difference between the two methods (MRI and DXA) is small. Bland Altman analysis showed a discrepancy between the change in muscle mass as determined by MRI and DXA in absolute terms of 0.09 kg and in percentage terms of 0.18% over the 5-year period. This suggests that DXA is an acceptable method for longitudinal tracking of muscle mass in older people.

Longitudinal age-related decline in muscle mass in older people

Ageing is associated with an overall reduction in skeletal muscle mass that contributes significantly to the loss of muscle strength (McPhee et al., 2018). This loss of strength and concomitant slowing of the muscle (Simunic et al., 2018) result in an age-related reduction in muscle power that is associated with a reduced performance in the timed-up-and-go and 6-minute-walking test (Maden-Wilkinson et al., 2015). As the proportion of older people is rising in the western world, it is important to understand sarcopenia and its progression towards frailty in the older person (McPhee et al., 2016). Here, we found with both DXA and MRI that over the relatively short time period of 5 years, muscle mass decreases by ~5% in people in their seventies. This is relatively more than the 25% lower muscle mass seen in a cross-sectional comparison of recreationally active people in their seventies and their 50 years younger counterparts in their twenties (Maden-Wilkinson et al., 2014). It indicates that the age-related rate of muscle decline is possibly accelerated during the eighth decade of human ageing (Degens and Korhonen, 2012, Ganse et al., 2018), and/or that the rate of loss of muscle mass before age 70 only starts beyond e.g. the age of 45 (Janssen et al.,

2000c). Thus halving the period of atrophy between the twenties and seventies. It is important that we are able to distinguish the differing phases of ageing, so we are able to describe changes in the loss of muscle mass seen at various points during the human lifespan.

Some studies report that the age-related loss of muscle mass is larger in men than in women (Iannuzzi-Sucich et al., 2002, Castillo et al., 2003, Janssen et al., 2000c), while others show similar losses for both genders (Janssen et al., 2002). Part of the discrepancy may be due to the way changes in muscle mass are reported. In absolute terms men lose more mass than women because men have a larger muscle mass to start with, but in percentage terms the decrease is similar for men and women, as we observed in the present 5-year longitudinal study. In line with this, we found that while baseline muscle mass was, if anything, associated with a larger loss of muscle mass, it did not correlate with the percentage age-related decline in muscle mass. It has been reported that a lower muscle mass is associated with functional impairment and physical disability (Janssen et al., 2002). Though in absolute terms, as there was no difference in the relative rate, the decrease in muscle mass occurs at a faster rate in those with larger muscles, they will reach the disability threshold later, illustrating that it is in the long run beneficial to have a larger muscle mass (Degens, 2018, Degens and McPhee, 2013)

Previous cross-sectional studies have shown that increased levels of adipose tissue may accelerate the age-related loss of muscle mass and strength in both men and women (Newman et al., 2003, Koster et al., 2011, Tomlinson et al., 2014, de Carvalho et al., 2018). In the present study the percentage muscle loss over 5 years was positively related to the percentage body fat in men, but this was not the case for women. Particularly visceral fat mass is an important source of inflammatory cytokines

(Pedersen, 2009) and an increase in the fat mass is likely to contribute to chronic low-grade systemic inflammation in older people that can cause muscle wasting and dysfunction (Degens, 2010). With visceral fat being shown to accelerate the loss of muscle mass in older individuals in particular (Kim et al., 2014). These observations stress the benefit of a low body fat percentage for skeletal muscle health in old age and hence the importance of a healthy diet and regular physical activity (McPhee et al., 2016, Mithal et al., 2013).

Previously, it has been observed in a cross-sectional study that there appears to be a differential rate of atrophy between extensors and flexors seen in ageing, with the quadriceps muscles declining by 30% and the other muscles in the thigh only declining 18% in older people in their seventies, compared to young-adults in their twenties (Maden-Wilkinson et al., 2013b). Here we did not see a differential rate in loss of muscle mass over the 5-year period. It remains to be seen whether there is indeed a differential rate of atrophy in these muscles during early ageing, followed by a similar rate of age-related atrophy in old age.

Conclusion

Both DXA and MRI showed a similar percentage atrophy over a 5-year period in septuagenarians. The rate of atrophy was independent of the muscle mass at baseline and similar for men and women. A high percentage body fat was, however, associated with a faster rate of muscle decline in men. These data indicate that 1) DXA can be used to assess longitudinal changes in muscle mass in older people, 2) a proportionally larger decline of muscle mass than the 25% difference between people in their twenties and seventies (Maden-Wilkinson et al., 2013b), 3) a low muscle mass

is not indicative of a higher rate of age-related muscle wasting and 4) increased body fatness was associated with an greater rate of age-related muscle loss in men.

Chapter 3

The Contributions of Fibre Atrophy, Fibre Loss, *In Situ* Specific Force, and Voluntary Activation to Weakness in Sarcopenia

3.1 Abstract

The contributions of fibre atrophy, fibre loss, *in situ* specific force and voluntary activation to weakness in sarcopenia remain unclear. To investigate, forty older (20 women; age 72±4yrs) and 31 younger adults (15 women, age 22±3yrs) completed measurements. The knee extensor maximal voluntary torque (MVC) was measured as well as voluntary activation, patella tendon moment arm length, muscle volume and fascicle architecture to estimate *in situ* specific force. Fibre cross-sectional area (FCSA), fibre numbers and connective tissue contents were also estimated from vastus lateralis biopsies. The MVC, quadriceps volume and specific force were 39%, 28% and 17% lower, respectively, in old compared with young, but voluntary activation was not different. The difference in muscle size was due in almost equal proportions to lower type II FCSA and fewer fibres. Five years later (*n*=23) the MVC, muscle volume and voluntary activation in old decreased an additional 12%, 6% and 4%, respectively, but there was no further change in specific force. Conclusions: *in situ* specific force declines relatively early in older age and reduced voluntary activation occurs later, but the overall weakness in sarcopenia is mainly related to loss of both type I and II fibres and type II fibre atrophy.

3.2 Introduction

Skeletal muscle weakness is a key feature of sarcopenia (Cruz-Jentoft et al., 2019) and a core component of the physical frailty phenotype (Fried et al., 2001). Weakness increases the effort required to complete everyday physical tasks and is associated with a higher risk of falling, disability, hospital admission and mortality (Clark and Manini, 2010). To develop effective countermeasures, it is important to understand the factors contributing to weakness.

In young adults, a close relationship exists between muscle cross-sectional area and the maximal force produced by that muscle (Bamman et al., 2000, Maughan et al., 1983). During ageing the muscle mass declines in part due to type II fibre atrophy (Andersen, 2003, Barnouin et al., 2017a), which contributes to muscle weakness. Fibre losses may also contribute to low muscle mass, although there is surprisingly little data on this matter and conflicting reports with one suggesting fibres are lost with ageing (Lexell et al., 1988) and another stating they are not (Nilwik et al., 2013). Irrespective of the reasons why muscle mass declines, recent reports argue that the relationship between muscle mass and maximal force is weak in older adults (Senechal et al., 2015, Clark and Manini, 2008). This viewpoint is based on the apparent disparity in the age-related changes of maximal force and lean mass seen in cross-sectional studies (Chen et al., 2013, Lynch et al., 1999) and longitudinal studies where a three-fold greater decline of maximal force compared to appendicular lean mass has been reported (e.g. (Goodpaster et al., 2006, Hughes et al., 2001a)). It might be concluded, therefore, that low muscle mass is not the primary cause of weakness in older age and this has led to interest in possible changes in “muscle quality”, measured as maximal force per unit muscle mass (e.g. see (Goodpaster et al., 2006, McGregor et al., 2014, Hairi et al., 2010b, Lynch et al., 1999, Moore et al., 2014)).

However, this literature has two important limitations. First, it is strongly influenced by studies using DXA to estimate the muscle size. Additionally, previous work did not consider potentially important physiological and anatomical contributions to force production, including activation of the motor unit pool, muscle architecture and joint structures.

To understand the causes of weakness in older age, it is necessary to take account of several factors. First, the maximal muscle force depends on all available motor units of the agonist muscles being fully activated (Clark and Taylor, 2011). Secondly, muscle force is proportional to the number of fibres (or, sarcomeres) in parallel, represented by the physiological cross-sectional area (PCSA), rather than the anatomical cross-sectional area (CSA), of the agonist muscles (Degens et al., 2009b). Thirdly, muscle and joint architecture influence the external torque because the tendon force decreases in proportion to the cosine of the fibre pennation angle, while external torque increases proportionately with the tendon moment arm (Degens et al., 2009b). Considering all of these factors together gives a better estimate of muscle quality than just normalising maximal force to lean mass derived from DXA, and is referred to here as *in situ specific force*. Previous studies showed lower *in situ* specific force of plantar flexors (Morse et al., 2005b) and voluntary activation of knee extensors (Clark and Taylor, 2011) in old compared with young. However, there is currently limited information about specific force and voluntary activation of sarcopenic muscle and no information about longitudinal changes in specific force for older adults.

The aim of the present study was to estimate the contributions of muscle size and specific force to the maximal external muscle torque in young and older adults. The hypothesis was that low muscle mass as well as reduced voluntary activation and *in situ* specific force contribute to weakness in sarcopenia. Following on from this, we

aimed to estimate the contributions of muscle fibre atrophy and muscle fibre loss to the overall quadriceps muscle atrophy with ageing. These aims were addressed through comparison of results from young and older adults and a longitudinal examination of older adults.

3.3 Methods

Ethical approval and research participants

The Local Research Ethics Committee approved the study. All volunteers provided written informed consent. Volunteers were excluded if they were involved in any competitive sports (recreational sports were allowed) or had cardiovascular (controlled hypertension was allowed), metabolic, musculoskeletal, neurological or mental conditions, or body mass index <18 or >32 $kg \cdot m^{-2}$.

Participants arrived at the research facility between 9am and 10am. DXA and MRI images were collected, followed by the grip strength, timed-up-and-go and 6-minute walk tests. A light snack and drink were provided and after a 30 minute break, the assessments of knee extensor voluntary activation and architecture were completed. For longitudinal studies, the older participants were invited to complete the same assessments 5 years later. Participant characteristics are shown in Table 1, including results for the basic functional assessments of 6 minute walk test (walking as far as possible in 6 min around cones placed 20 m apart), maximal grip strength (Jamar dynamometer performed twice on each hand and the maximum value taken) and timed up and go (TUG: starting from a seated position, stand and walk around a cone placed 3 m in front and then return to the original seated position), which were all performed following standardised procedures described previously (McPhee et al., 2013).

Musculoskeletal imaging

Participants were scanned by DXA (Lunar prodigy advance, GE Healthcare, Chalfont St Giles, UK) after an overnight fast in the supine position whilst wearing a light cotton robe. Off-line analysis (encore 2006 v 10.50.086) identified whole body lean mass and body fat percentage, arm and leg lean mass and bone mineral content (McPhee et al., 2013). ALM was calculated as: [(lean mass of legs + lean mass of arms) – (bone mineral content of legs + bone mineral content of arms)] (Goodpaster et al., 2006).

A 0.25-T MRI scanner (G-scan, Esaote Biomedica, Genoa, Italy) was used to collect transverse plane sections (Turbo-3D T1-weighted protocol with consecutive 2.8 mm thick slices) from the dominant leg tibial tubercle through to the anterior-inferior iliac spine with participants in the supine position (Maden-Wilkinson et al., 2013a). Osirix imaging software was used to estimate the anatomical cross-sectional areas of each of the four heads of the quadriceps muscles from transverse-plane images at 25 mm intervals from the distal to the proximal ends of the quadriceps. These cross-sectional areas were summed and multiplied by the distance between slices (2.5 cm) to estimate quadriceps muscle volume. The patella tendon moment arm was imaged with the leg at full extension and estimated from sagittal-plane slices as the distance from the mid-contact point between the femoral condyles and tibial plateau to the patella tendon. The moment arm length was multiplied by 0.99 to adjust for the difference between full knee extension to 90° flexion (Baltzopoulos, 1995) (the angle at which MVC was measured) and the resulting value was multiplied by 1.14 to adjust for the 14% increase in moment arm in transition from rest to MVC (Tsaopoulos et al., 2007). This technique has a coefficient of variation of < 4% (Erskine et al., 2009).

Grip Strength

Handgrip strength was measured with all participants instructed to maintain an upright standing position, arms down by the side, holding the dynamometer in the dominant hand without squeezing the arm against the body. Measurement was to the nearest 0.1kg using a JAMAR hand dynamometer (Sammons Preston, Inc, Bolingbrook, IL, USA). Each participant had the dynamometer adjusted to their hand size, with the 2nd phalanx resting against the inner handle.

Six-Minute Walk Distance

To assess the 6-minute walk distance two cones were placed 20 m apart. Participants were given the verbal instruction to “complete as many circuits as possible without running” and received verbal encouragement after each minute of the walk. The total distance walked during the six-minute period was recorded (Enright, 2003). Heart rate was monitored throughout the test (Polar, USA) and the average heart rate during the final 3 minutes of the test was given as the steady state heart rate (S-shr). All participants completed the 6-minute walk without the use of a walking aid.

Timed Up-and-Go

The TUG test involved getting up from a standardised chair (no arm rests, seat 44 cm high) and to walk forward as quickly as they were able, without running, to a cone 3 m away and return to the initial sitting position. Participants were familiarised to the procedure prior to the execution of the real test. Upon the ‘go’ signal, participants rose from the chair and timing was concluded when seated again. The test was conducted three times for each participant, with a rest period of 1 min between trials, and the quickest of the three trials was recorded.

MVC and voluntary activation

A custom-built isometric dynamometer was used to assess knee extension MVC of the dominant leg with participants sitting with the knee and hip angles at 90°. A strap was firmly secured across the hip joint and the lower leg securely strapped to the force transducer 2 cm above the malleolus. The linear distance from the estimated centre of knee rotation to the point of force application (2 cm above the malleolus) was taken as the lever length (in m). Torque was estimated as force multiplied by lever length. Force signals sampled at 2000 Hz were digitised for real-time visual display and for recording on a computer interface running Labview and a customised Matlab script (Matlab, the Mathwork Inc., S Natik, MA, USA). Participants were familiarised with the knee extension exercise by performing up to five contractions at around 50% of maximal effort each lasting 3 s, and another two contractions at around 80% maximal effort. After a 2 min rest, participants performed a maximal isometric contraction, sustained for 3 s with visual feedback and strong verbal encouragement and this was repeated a further two times. The highest recorded torque was taken as MVC. The patella tendon force was estimated from the moment equilibrium equation around the knee joint (Reeves et al., 2004a) by dividing the MVC torque by the patella tendon moment arm length.

Voluntary activation was assessed using a version of the interpolated twitch technique (Rutherford et al., 1986b, McPhee et al., 2014) with stimulating electrodes covering the proximal and distal portions of the quadriceps (AmericanImex: Dispersive electrode, 4 x 7 inch), connected to a Digitimer DS7AH set at 400 V (Welwyn Garden City, UK) and current increased to deliver supramaximal ‘doublet’ (two 200-μs pulses separated by 10 ms) stimuli over the quadriceps muscle group. Stimulation was applied to the relaxed muscle 1 s prior to a maximal voluntary effort and then again at

the highest point of the MVC. In the cross-sectional study, a third doublet was also applied 2 s after the MVC. The voluntary activation test was performed twice and the result giving the highest voluntary activation was accepted. The percentage voluntary activation was calculated as:

- % voluntary activation = 100 x (1 – t/T)

Where t was the amplitude of the superimposed doublet (i.e. the size of the additional peak) and T the value of the doublet applied to the resting muscle 1 s prior to MVC.

Physiological cross sectional area and *in situ* specific force

PCSA was calculated for each quadriceps muscle as: [muscle volume / fascicle length], and the sum taken as quadriceps PCSA (Narici, 1999).

The fascicle length and pennation angle used in these calculations were estimated using real-time B-mode ultrasonography with a 7.5-MHz linear array probe. Measurements were collected at the mid belly of each of the four heads of the quadriceps muscles in the sagittal plane at the moment of peak force during MVC contractions (Erskine et al., 2009). Imaging software (Image J; v1.39b; National Institutes of Health, Bethesda; USA) was used to determine muscle fascicle length from the superficial to the deep aponeurosis. Pennation angle was determined as the angle at which the fascicles intercepted the deep aponeurosis. Thickness was measured as the perpendicular distance between the superficial and deep aponeurosis.

For calculations of *in situ* specific force, the quadriceps PCSA was multiplied by the cosine of the fascicle pennation angle to account for the reduction in transmission of forces from fibres to aponeurosis to adjust for the angle between the fascicles and the line of pull through the patella tendon. Specific force was estimated as: [(external torque / moment arm) / (PCSA * pennation angle)] *or* simplified to: [Patella tendon

force / (PCSA * pennation angle)] (Erskine et al., 2009). The external force used in the calculation of specific force was the *"true MVC",* which is the estimated MVC if full voluntary activation was possible: *True MVC* = MVC immediately prior to the superimposed doublet / (1 – t/T). Where t was the amplitude of the superimposed doublet (i.e. the size of the additional peak) and T the value of the doublet applied to the resting muscle 1 s prior to MVC.

Muscle morphology

Muscle biopsies available from young and old participants were taken using a conchotome from midway along the length of the right vastus lateralis muscle (VL) under aseptic conditions and local anaesthesia (1% lignocaine). The samples were placed on cork with fibres running vertically and immediately frozen in liquid nitrogen whilst shaking vigorously to avoid freezing artefacts. Muscle sections were stained for myosin ATPase activity after preincubation at pH 4.35 to identify type I and type II fibres and determination of the fibre cross-sectional areas (FCSA). Serial sections were stained with Sirius Red to assess the collagen content and analysed using a customised Matlab programme. The total numbers of fibres in the VL PCSA was estimated as: [VL PCSA / average FCSA]. Biopsies were not collected in the longitudinal follow-up study.

Statistical analysis

The Shapiro-Wilk test showed that all data were normally distributed. A two-way ANOVA was used to test for age and gender effects of outcome parameters. Pearson's Product Moment Correlations were used to assess the relationship between variables. Changes occurring over the 5-year follow-up period were assessed using paired samples t-tests. Two stepwise multiple regression models were used to identify factors associated with MVC torque: the first for baseline and the second for follow-up

changes. Both included quadriceps volume, voluntary activation, VL fascicle pennation angle, patella tendon moment arm length, age and gender. The second model was adapted to examine predictors of longitudinal changes in MVC torque and used the percentage changes in each of these variables. Standardised beta coefficients (β) indicate the change in standard deviation of MVC torque per standard deviation change in the independent variable. Statistical testing was completed using SPSS (IBM v.23. USA) and significance was accepted as $P<0.05$. Results are reported as mean and standard deviation (SD), unless otherwise stated.

3.4 Results

Table 3.1. *Participant characteristics*

	Baseline study of young and older adults							Longitudinal study of older adults			
	YM (n = 16)	YW (n = 15)	OM (n = 20)	OW (n = 20)	*P* value: Gender	*P* value: age	O vs Y (%)	Baseline (n = 23)	Follow-up (n = 23)	*P* value:	Change (%)
Age (yrs)	23 ± 4	22 ± 2	72 ± 5	71 ± 4				71 ± 4	76 ± 4		
Height (m)	1.79 ± 0.06	1.67 ± 0.06	1.74 ± 0.08	1.60 ± 0.07	<0.001	0.001	-3	1.68 ± 0.10	1.67 ± 0.10	0.001	-1
Body mass (kg)	70.6 ± 8.3	61.2 ± 10.7	78.9 ± 14.4	67.3 ± 12.0	0.008	0.014	11	73.2 ± 14.8	73.4 ± 16.1	0.730	0
BMI ($kg \cdot m^{-2}$)	21.3 ± 2.2	21.6 ± 3.6	25.9 ± 2.8	26.3 ± 4.1	0.625	<0.001	19	26.0 ± 4.8	26.4 ± 4.7	0.126	1
Body fat (%)	16.2 ± 6.6	29.6 ± 8.4	30.2 ± 7.8	39.7 ± 8.3	<0.001	<0.001	47	32.6 ± 10.4	33.5 ± 10.1	0.049	3
ALM (kg)	24.2 ± 1.9	15.1 ± 1.9	20.9 ± 4.0	13.1 ± 2.1	<0.001	<0.001	-13	19.9 ± 5.0	19.3 ± 4.7	<0.001	-3
$ALM \cdot h^{-2}$ ($kg \cdot m^{-2}$)	7.6 ± 0.6	5.4 ± 0.5	6.9 ± 0.8	5.1 ± 0.7	<0.001	0.005	-8	6.9 ± 1.0	6.8 ± 0.8	0.070	-1
Grip strength (kg)	48.6 ± 12.1	34.3 ± 6.6	37.6 ± 7.7	25.3 ± 4.5	<0.001	<0.001	-24	32.2 ± 9.0	33.1 ± 7.9	0.799	3
TUG (s)	3.9 ± 0.4	4.2 ± 0.3	5.1 ± 0.8	5.6 ± 1.0	0.083	<0.001	32	5.2 ± 0.7	6.6 ± 1.2	<0.001	27
6 min walk (m)	735 ± 40	683 ± 45	562 ± 60	551 ± 87	0.081	<0.001	-21	563 ± 79	507 ± 69	<0.001	-10

Data shown as mean ± SD. *Abbreviations*: YM: young men; YW; young women; OM: older men; OW: older women. BMI: body mass index; ALM: appendicular lean mass; TUG: timed-up-and-go.

Muscle size, strength and specific force

Table 3.1 provides the data for grip strength, TUG and 6-min walk, as well as ALM and ALM/h^2 and the data show the older adults to be sarcopenic (Cruz-Jentoft et al., 2019). There were no significant age x gender interactions for any of the measurements, indicating that the effects of age described here apply similarly to men and women.

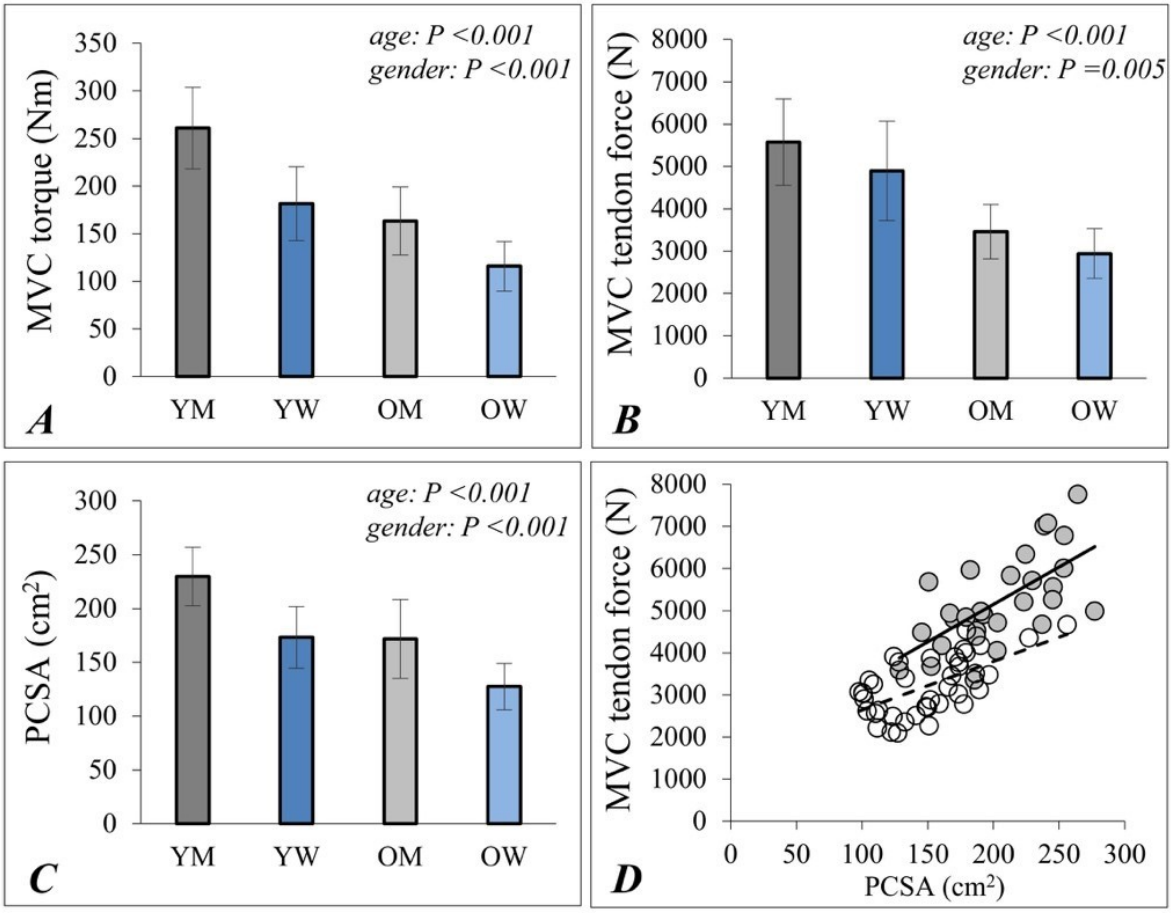

Figure 3.1. Knee extensor size and strength measurements in young and older men and women**.** A) knee extensor MCV torque; B) MVC patella tendon force; C) quadriceps physiological cross-sectional area (PCSA); D) the relationship between patella tendon force and quadriceps PCSA for young (shaded circles, continuous line) and older (filled circles, dashed line) adults. Data shown as mean ± SD (Figs 3.1A-C) and individual data points (Fig 3.1D). Abbreviations: MVC: maximal voluntary torque; PCSA: quadriceps physiological cross sectional area; YM: young men; YW: young women; OM: older men; OW: older women. *P* values indicate the results of a two-way ANOVA; age x gender interactions were not significant for any of these measurements.

The MVC torque (Figure 3.1A) and peak patella tendon force (Figure 3.1B) in old were 37% and 39%, respectively, lower than values for young. Quadriceps PCSA was 25% lower in old than young (Figure 3.1C) and was positively correlated (R^2=0.598; *P*<0.001) with tendon force (Figure 3.1D). The *in situ* specific force value for old was 83% of values for young (Figure 3.2; *P*<0.001).

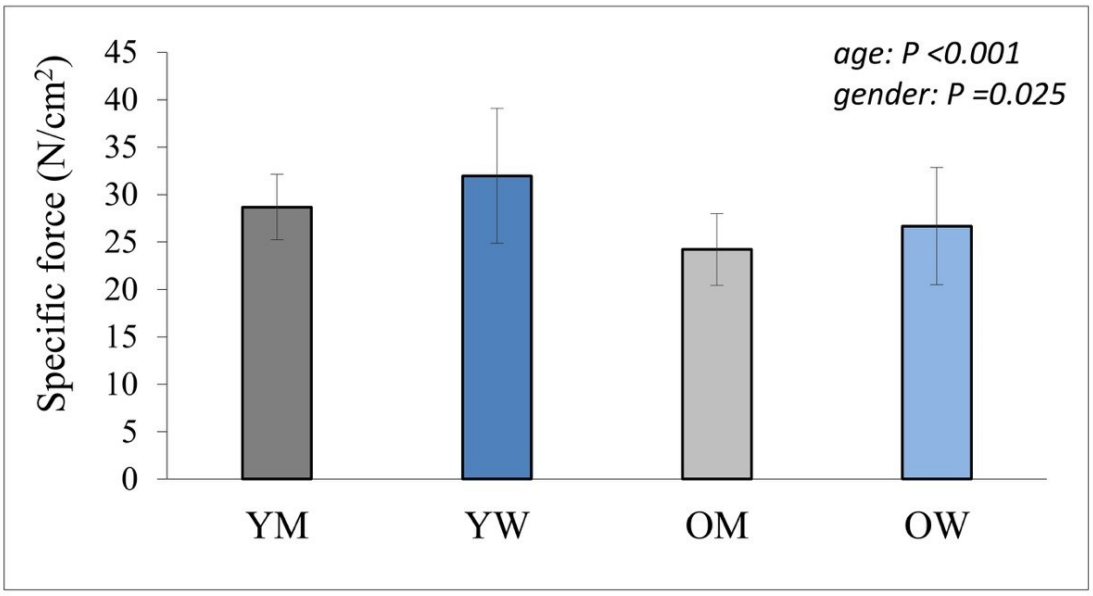

Figure 3.2. Knee extensor *in situ* specific force. Data shown as mean ± SD. YM: young men; YW; young women; OM: older men; OW: older women. *P* values indicate the results of a two-way ANOVA; there was no significant age x gender interaction.

During the 5 years of follow up, components of sarcopenia including ALMM and performance in the TUG and 6-min walk tests all decreased (Table 3.1). The percentage decrease from baseline values included 12% (±13) lower MVC torque, 6% (±9) lower quadriceps muscle volume, 5% (±9) lower PCSA and 4% (±6) lower

voluntary activation (all *P*<0.05), but the *in situ* specific force did not change significantly (3% (±11); Figure 3.3).

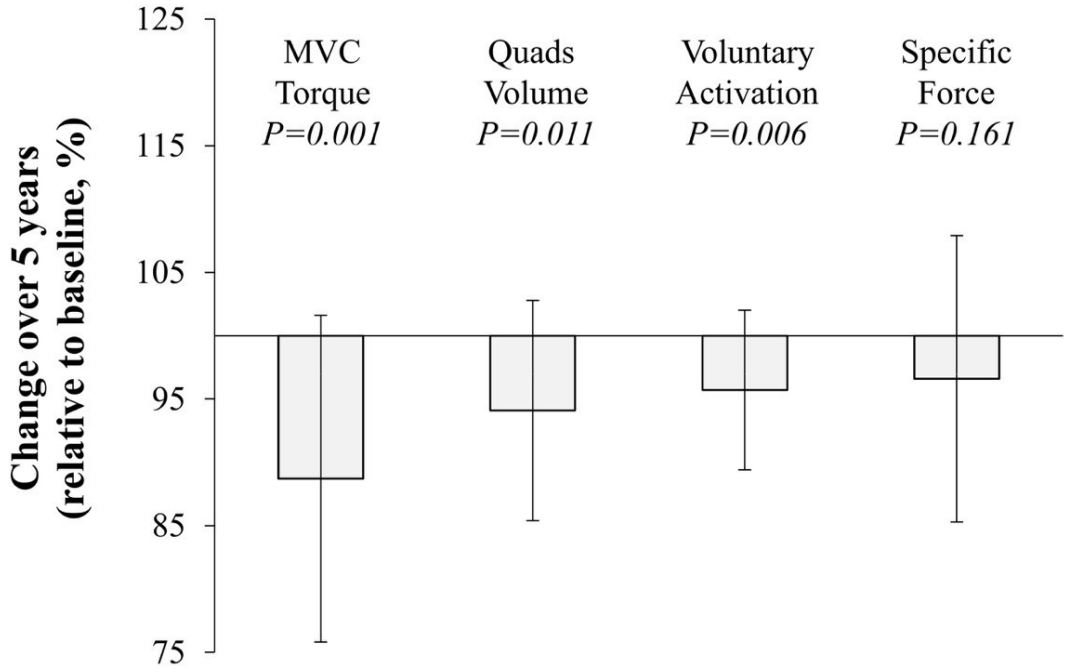

Figure 3.3. Five-year changes to muscle torque, size, activation and specific force. Data from older adults only and shown as mean ± SD. * indicates significant change from baseline

Predictors of MVC torque

The regression model based on data from young and old explained 83% of the variation in external torque. The majority was due to quadriceps volume (adjusted R^2=0.765; β=0.727; *P*<0.001) and a small contribution of age (adjusted R^2=0.057; β=-0.286; *P*<0.001). Gender, pennation angle, voluntary activation and moment arm length did not contribute significantly to the model. A similar result was found if quadriceps PCSA was used in the model rather than quadriceps muscle volume. The remaining 17% of the variation in external torque not explained by any variables in this model includes the contribution of muscle specific force.

The regression model based on longitudinal data explained 72% of the change in external torque, with the main factor being change in quadriceps volume (adjusted R^2=0.510; β=0.730; *P*<0.001) and a contribution of change in voluntary activation (adjusted R^2=0.210; β=-0.460; *P*=0.001). Gender, age, pennation angle and moment arm length did not contribute significantly to the model.

Muscle fibre cross sectional area and estimated fibre numbers per PCSA

Since the results comparing young with old and the longitudinal study point towards loss of muscle mass being the main determinant of low MVC in older age, additional analysis was completed to determine the relative contributions of fibre atrophy and fibre loss to the difference between young and old in VL PCSA.

The relative area occupied by type I and type II fibres did not differ significantly between young and old (*P*=0.423) or between men and women (*P*=0.726) (type I: young men: 38.8 ± 11.7; young women: 37.3 ± 8.8; older men: 42.0 ± 12.8; older women: 40.8 ± 7.4%).

Table 4.2. *Skeletal muscle characteristics*

	YM (n = 16)	**YW** (n = 15)	**OM** (n = 20)	**OW** (n = 20)	***P* value: Gender**	***P* value: age**	**O vs Y (%)**
Voluntary activation (%)	90.1± 3.7	92.2 ± 3.8	88.5 ± 5.9	90.9 ± 5.1	0.073	0.252	-1
Moment arm (cm)	4.0 ± 0.2	3.3 ± 0.3	4.0 ± 0.3	3.4 ± 0.3	<0.001	0.447	3
R. leg lean mass (kg)	10.3 ± 1.0	6.5 ± 0.8	8.8 ± 1.6	5.6 ± 0.8	<0.001	<0.001	-14
R. Quads mass (kg)	2.2 ± 0.3	1.4 ± 0.2	1.6 ± 0.3	1.1 ± 0.2	<0.001	<0.001	-28
VL fascicle length (mm)	90.6 ± 11.3	86.6 ± 12.0	95.0 ± 13.9	88.4 ± 10.1	0.073	0.290	4
VL Pennation angle (deg)	15.3 ± 2.9	13.8 ± 1.8	12.3 ± 2.1	11.9 ± 1.4	0.061	<0.001	-17
VL Thickness (mm)	25.2 ± 3.2	19.9 ± 2.9	20.4 ± 3.5	17.9 ± 2.8	<0.001	<0.001	-15
VI fascicle length (mm)	100.5 ± 11.0	92.5 ± 10.5	93.3 ± 12.6	87.4 ± 11.2	0.014	0.028	-6
VI Pennation angle (deg)	12.0 ± 2.5	10.9 ± 2.0	11.9 ± 2.7	10.5 ± 1.7	0.025	0.699	-2
VI Thickness (mm)	21.1 ± 4.9	18.2 ± 4.0	19.0 ± 3.1	15.9 ± 3.6	0.002	0.026	-11
RF fascicle length (mm)	76.7 ± 16.0	70.7 ± 10.8	78.3 ± 20.0	71.9 ± 19.7	0.150	0.738	2
RF Pennation angle (deg)	19.2 ± 4.0	18.0 ± 2.4	15.6 ± 3.1	14.9 ± 2.7	0.084	<0.001	-20
RF Thickness (mm)	23.6 ± 4.2	19.9 ± 4.0	20.4 ± 4.3	17.8 ± 5.0	0.005	0.015	-12
VM fascicle length (mm)	94.7 ± 13.1	74.1 ± 15.9	87.5 ± 12.0	74.9 ± 17.7	<0.001	0.375	-4
VM Pennation angle (deg)	20.8 ± 4.3	19.5 ± 7.2	19.6 ± 3.2	15.3 ± 2.7	0.011	0.014	-13
VM Thickness (mm)	31.3 ± 5.1	21.5 ± 3.4	26.6 ± 4.2	19.3 ± 4.8	<0.001	0.002	-13
Type I FCSA (μm^2)	4880 ± 690	4180 ± 920	5230 ± 1940	4160 ± 1190	0.708	0.487	4
Type II FCSA (μm^2)	6110 ± 1330	4600 ± 650	5000 ± 1440	2960 ± 500	<0.001	<0.001	-26

Data shown as mean ± SD. *Abbreviations*: YM: young men; YW; young women; OM: older men; OW: older women; FCSA: fibre cross-sectional area (available from 13 young men, 8 young women, 20 older men and 10 older women); VL: vastus lateralis; VI: vastus intermedius; RF: rectus femoris; VM: vastus medialis.

There was no significant difference between young and old in type I FCSA (P=0.487), but type II fibres had 26% lower FCSA in old (P<0.001; Table 3.2). Considering the type I and type II fibres together, the overall FCSA was 15% lower in old than young, which would give approximately 9.5 cm^2 smaller VL PCSA in old than young. However, the actual VL PCSA was 17.5 cm^2 (28%) smaller in old than young, suggesting that fibre atrophy alone accounts for approximately 54% of the overall muscle atrophy and the remainder (46%) is due to old having fewer fibres than young. The estimated numbers of muscle fibres per VL PCSA (PCSA divided by FCSA) was 1.22 million in young and 1.03 million (15% fewer) in old. The old had a higher proportion of connective tissue than young (11.3 ± 1.0% in young and 14.2 ± 1.4% in old). Taking into account this 3% difference reduces the estimated number of fibres per VL PCSA to 1 million in old, which is 18% fewer than the young.

3.5 Discussion

We have considered muscle quantity, quality and activation to understand the causes of muscular weakness (sometimes referred to as dynapenia, from the Greek for poverty of strength) in sarcopenia. The results show that 28% lower muscle mass was the main cause of weakness in old compared with young and a further decrease in muscle mass was the main predictor of progressing weakness over the follow-up period. *In situ* specific force was 17% lower in old compared with young and did not decrease further during the follow-up period. Voluntary activation was similar for young and old, but the 4% decrease over five years of follow-up contributed to the declining MVC torque. The lower muscle mass in older age was due in about equal proportions to fibre atrophy and loss of fibres.

Knee extensor torque and size

Low muscle mass is the criterion measurement for sarcopenia and can be estimated as ALM/h^2. The average ALM/h^2 of 6.9 kg/m^2 for older men and 5.1 kg/m^2 for older women at baseline were below the recommended sarcopenia cut-off values of 7.26 kg/m^2 for men and 5.5 kg/m^2 for women (Cruz-Jentoft et al., 2019) . Components of sarcopenia further declined at follow-up (Table 4.1). In the results comparing young with older adults there was a 26-28% lower muscle size (PCSA and volume, respectively) and 37% lower MVC torque. If we assume muscle declines begin from age 30 years (Janssen et al., 2000b, Moore et al., 2014, Lynch et al., 1999, Silva et al., 2010), the rate of change is estimated to be 0.9, 0.7 and 0.4% per year, respectively, for MVC torque, quadriceps size and *in situ* specific force. A further 12% decline in MVC was observed over the 5 years follow-up of the older adults. This rate of decline is more than twice that estimated for the previous 40 years and is generally

in agreement with the literature highlighting accelerated deterioration with advancing older age (Mitchell et al., 2012).

Our results suggest that the cause of muscle weakness during ageing to around age 70 years is due to loss of muscle mass and, to a lesser extent, specific force. An important novel finding of the present study was that the further weakening into the late 70s is primarily attributable to continued decline of muscle mass and a lower voluntary activation.

In situ specific force

Recent reports suggest that muscle quality is the major determinant of strength in older age (Senechal et al., 2015, Clark and Manini, 2008). This literature is largely based on DXA studies to estimate lean mass, where only weak relationships are seen with MVC torque or force (Senechal et al., 2015, Clark and Manini, 2008, Lynch et al., 1999). These studies using DXA do not measure the agonist muscle size and in this respect, the MRI is the criterion technique and CT is also preferable to DXA (Lustgarten and Fielding, 2011). Our results using MRI do not support the literature stating a large discordance between muscle mass and strength in older adults. Rather, a positive relationship exists between quadriceps PCSA and patella tendon force in young and old (Figure 4.1), which is in keeping with the long-standing literature from MRI and CT imaging (Hakkinen and Hakkinen, 1991, Maughan et al., 1983, Young et al., 1984, Overend et al., 1992, Rutherford and Jones, 1992, Bamman et al., 2000).

A large-scale longitudinal observation of muscle quality that included an accurate (CT) measurement of muscle size showed around 5% loss of thigh muscle CSA and 16% decrease in MVC force over 5 years in older men and women (Delmonico et al., 2009b). This disproportionate loss of strength compared to mass was interpreted as reduced muscle quality being the decisive factor for weakness (Delmonico et al.,

2009b). However, without a measurement of the neural activation, these findings alone should not be interpreted in this way. Because strength can decline due to subjects being less willing to perform a maximum contraction or less able to activate the motor unit pool, as has been demonstrated in our results and others have previously shown (Clark and Taylor, 2011, Harridge et al., 1999). We calculated voluntary activation using the superimposed doublet normalized to the pre-MVC doublet, based on the assumption that the superimposed stimulus activates motor units (muscle fibres) not recruited during the voluntary effort. Other studies normalized the superimposed stimulus to a stimulus applied 2 s after the MVC when the muscle response can be potentiated. Our cross-sectional study dataset includes both the “pre-stimulus” and the “post-stimulus” so we were able to compare the results. The average voluntary activation calculated for all participants pooled was 90.4% and 90.8% ($p<0.001$) when using the “pre-stimulus” and the “post-stimulus”, respectively. It therefore made no difference to results if the pre- or the post- stimulus was used to normalize the superimposed stimulus.

The best estimate of the muscle quality comes from measurement of the *in-situ* specific force, which takes into account the agonist muscle size, architecture, activation of the motor unit pool and the patella tendon moment arm length. Our results show 17% lower specific force in old compared with young, which is similar to the findings of a previous study normalizing knee extensor isokinetic MVC to quadriceps anatomical cross-sectional area (Jubrias et al., 1997). We observed no significant change in specific force over the 5-year follow-up in older adults. This is the first longitudinal study of *in situ* specific force accounting for patella tendon force, PCSA and muscle architecture. One previous study measured specific force in a similar way to us, but comparing young and older plantar flexors. They reported that a 37% lower

Achilles tendon force in older muscle was mostly due to a lower (30%) specific force (Morse et al., 2005b). Their conclusion that muscle quality changes are more important than muscle quantity differs from our own, but closer inspection of the published results (Morse et al., 2005b) also reveals 28% lower muscle volume (Morse et al., 2005b), which is in fact in agreement with our own findings that changes in muscle quantity are playing the largest role in age-related weakness.

The results of the present and a previous publication (Erskine et al., 2009) reveal that the force / anatomical cross-sectional area (ACSA) gives a very similar age-related difference as the more comprehensively measured *in situ* specific force. *In situ* specific force is calculated as: [Patella tendon force / (PCSA * pennation angle)], where the patella tendon force is the force that could be produced if full voluntary activation was possible. Since the moment arm and the voluntary activation did not differ between young and old, the tendon force decreased proportional to that of torque. Furthermore, the lower PCSA in old was mainly due to a change in muscle volume because the fascicle length was similar for old and young and age-dependent differences in pennation angle have minimal influence on force transmission to the tendon. Thus, the age-dependent differences for *in situ* specific force are reasonably estimated from force / ACSA.

In mice (Ballak et al., 2014), connective tissue accumulation was associated with lower specific force. The small increase in connective tissue in old compared with young in our study explains at best just 3% of the difference between young and old in specific force, since connective tissue makes up a relatively small proportion of the overall muscle. The lower specific force is likely due to lower specific tension of individual muscle fibres in old compared with young (D'Antona et al., 2003, Frontera et al., 2000b, Brocca et al., 2017). We recently reported 16% lower single fibre specific

tension in old compared with young (Brocca et al., 2017) and this matches the estimates of the *in situ* specific force made in the present study.

The age-dependent muscle atrophy

Our results are consistent with previous reports that type II fibres are highly susceptible to age-related atrophy, while type I FCSA is well preserved (e.g. see (Barnouin et al., 2017a, Andersen, 2003, Nilwik et al., 2013, Lexell and Taylor, 1991)). However, surprisingly little information is available about muscle fibre numbers in humans and the scarcity of information limits current understanding of the contributions of muscle fibre changes underpinning the overall atrophy. In the present study, fibre atrophy accounted for 54% of the difference between the PCSA of young and old. The remaining 46% is presumably due to fibre losses and we estimate that the old had around 200,000 fewer fibres than young in the VL cross section. Despite the selective type II atrophy, the relative area occupied by type I and type II fibres did not differ between young and old, which must mean that a greater number of type I fibres than type II is lost to balance the reduction in type II FCSA. These findings are in general agreement with data from autopsy examinations of the VL muscle, suggesting that loss of fibres and type II fibre atrophy both contribute to the loss of VL muscle mass with ageing, although the autopsy studies indicate similar proportional losses of type I and type II fibres (Lexell et al., 1988, Lexell and Taylor, 1991).

A different conclusion was reached by Nilwick et al (2013), who reported that young and old men had similar fibre numbers and that loss of muscle mass with ageing was due to type II fibre atrophy only. Notably, Nilwick et al's (2013) older subjects were not sarcopenic and thus had much larger quadriceps anatomical cross-sectional area (QACSA: 59 cm^2 in our subjects and approximately 68 cm^2 in Nilwik et al. (2013)), but similar FCSA and therefore higher fibre numbers than our older, sarcopenic

participants. These differences between studies may reflect differences between sarcopenic and non-sarcopenic old, or differences in the ageing process due to lifestyle and habitual activity patterns.

Our estimate of 200,000 fewer fibres in the VL cross-section of old compared to young is similar to Lexell et al (1988) who estimated about 264,000 fewer fibres in septuagenarians compared with young adults. Lexell's (1988) data show fibre numbers decline after age 30 years, which is the same age that muscle mass begins to decrease (Janssen et al., 2000b, Moore et al., 2014, Lynch et al., 1999, Silva et al., 2010). It is highly likely that the other quadriceps muscles age in a similar way based on the fact that the different quadriceps muscles experience the same degree of atrophy (Maden-Wilkinson et al., 2013a) and undergo similar motor unit remodelling (Piasecki et al., 2016a, Ling et al., 2009). Given that the VL accounts for about 30% of the quadriceps mass, it can be estimated that 20,000 fibres are lost in each quadriceps muscle per year, or 40,000 fibres across both quadriceps muscles per year, from age 30 years, assuming linear progressive declines. The loss of fibres may be linked to declining numbers of motor units, as old have 30-50% fewer leg motor neurons than young adults (Tomlinson and Irving, 1977, Piasecki et al., 2016a). A resistance training programme will help to recover the type II fibre atrophy (Nilwik et al., 2013, Doherty, 2003b) and improve specific force (Reeves et al., 2004a), but is unlikely to recover lost fibres or motor units.

Conclusions

The *in situ* specific force declines relatively early during ageing and reduced voluntary activation of muscle occurs later, but the overall weakness in sarcopenia is mainly related to loss of both type I and type II muscle fibres and type II fibre atrophy.

Chapter 4

Decrements of mobility and power in septuagenarians related to loss of force, but not slowing of the muscle; a 5-year longitudinal study

4.1 Abstract

Previous work has found that the lower 6-minute walk distance (6MWD) was primarily due to intrinsic slowing of the muscle between young and old (Maden-Wilkinson et al., 2015). Here we investigated the ageing-related reductions in mobility over a 5-year period in septuagenarians. We measured muscle power by a countermovement jump, MVC, quadriceps muscle size by MRI in 17 older women (71.1±2.8 y) and 17 older men (71.3±4.1y). 6MWD and TUG were used as indicators of ability to perform daily life tasks. Performance in TUG and 6MWD were reduced in both genders ($P<0.001$). TUG and 6MWD correlated with power at both baseline and follow up ($R\geq0.53$; $P\leq0.001$). Of the components of power, jump take-off velocity (V_{CMJ}) correlated with 6MWD and TUG ($R\geq0.54$; $P\leq0.001$). However, the relationship between 'body mass: maximal force ratio' with V_{CMJ} was not significantly changed, the lower V_{CMJ} was attributable to the muscles working at a higher relative load, hence a lower part of the force-velocity relationship, due to a reduction in MVC rather than slowing of the muscle. In conclusion, the additional decrement in 6MWD & TUG in older people is due to a loss of strength rather than further slowing of the muscle.

4.2 Introduction

Globally, the proportion of people aged over 60 years is now growing rapidly. In 2012 there were already an estimated 810 million people over the age of 60, which is expected to rise to 2 billion by 2050 (United-Nations, 2012). A large proportion of older people suffer from limited mobility and loss of independence, placing a strain on healthcare resources. The ageing-related reductions in musculoskeletal function (dynapenia) and mass (sarcopenia) contribute to decreased mobility, and may ultimately lead to loss of independence and quality of life (McPhee et al., 2016). Counteracting or slowing musculoskeletal ageing might thus help to ease the burden. There are suggestions that the rate of ageing differs between people (Belsky et al., 2015, Pollock et al., 2015), possibly due to differences in genotype, physical activity, lifestyle and diet (Degens and Korhonen, 2012), which raises the possibility of an optimal model of ageing.

Athletic prowess (force and velocity) are thought to peak during the mid-20's (Rittweger et al., 2009, Berthelot et al., 2012). Upon entering the 4th decade there is a noticeable and constant decline in performance and strength (Janssen et al., 2000c) with a possible accelerated decline after the age of 70 (Hughes et al., 2001b, Ganse et al., 2018, Nikolaidis, 2018). Close associations between declining muscle mass and functional performance have been widely reported (e.g. (Bijlsma et al., 2014, Janssen et al., 2002), suggesting a causal relationship. However, changes in muscle mass may not be the entire explanation since muscle force and power has been reported to decline proportionally more than muscle size with age (Morse et al., 2005a, Degens et al., 2009b, McPhee et al., 2018). Indeed, previous studies have shown that muscle force and power are much more closely related to the ability to perform activities of

daily living than muscle mass in older people (Buford et al., 2012, Maden-Wilkinson et al., 2015, Bean et al., 2002, Reid and Fielding, 2012).

In addition to changes in muscle strength or power there are other factors that change with age and can affect performance of tasks related to mobility, such as a decline in neuromuscular coordination and cardiovascular function. Indeed, it has been shown that the performance in a 6MWT is related to the maximal oxygen consumption and performed at 80-86% of the measured maximal heart rate (Manttari et al., 2018). The ageing-related reduction in maximal heart rate (Tanaka et al., 2001) can thus contribute to a reduction in the performance in the 6-minute walk test.

In 2015 the results of a cross-sectional study of young adults (average age 23 years) and older men and women (average age 72 yrs) were published (Maden-Wilkinson et al., 2015). In that study there was evidence of an association between the lower power, determined from a standing jump, and lower TUG and 6MWD performance, that was primarily attributable to intrinsically slower muscles of the old than young-adult people. Five years following the end of the aforementioned cross-sectional study, this study repeated the measurements on a sample of the original older study population. Assessing the changes that had occurred during this five-year period, and to what extent any additional reduction in performance of the TUG and 6MWD in recreationally active older people is attributable to further slowing and/or weakening of the muscle. We hypothesised that 1) the annual rate of decline in TUG, 6MWD and muscle function over 5 years in septuagenarians will be larger than that seen between 23 and 72 years of age, 2) any additional decrement in performance will be related to a further intrinsic slowing of the muscle. In addition, we explored possible effects of ageing-related changes in cardiovascular function and balance on these functional measures.

4.3 Methodology

Participants and ethical approval

The study received approval from the local ethics committee and was performed in accordance to the declaration of Helsinki. Participants were recruited from a subgroup in the framework of the MYOAGE study (www.myoage.eu) (McPhee et al., 2013). Thirty-five participants returned 5 years following the initial cross-sectional study from 2009-2012 (base line). The follow up study was conducted between May 2015 and October 2015. The data of one woman are presented in figures, but not included in statistical analyses as her performance in the 6-minute walk and timed-up-and-go tests at follow up was more than 3 standard deviations below the average performance of the women at follow up (see her performance indicated with arrows in Fig. 1 & 2).

The characteristics of the included participants are presented in table 1. Written informed consent was obtained at base line and for the follow up study from each participant. Exclusion criteria were: institutionalisation, unable to complete 250-m walking unassisted, co-morbidities such as heart failure, chronic pain syndrome, metabolic disease, chronic obstructive pulmonary disease and/or neurological disorders (e.g. Parkinson's). Participants were also excluded if they had undergone hip or knee replacement in the previous 2 years, or had been immobilised for greater than 1 week 3 months prior to testing. All the participants were community dwelling and socially active. Participants were not known to suffer from musculoskeletal or cardiovascular disease, any limb fractures within 5 years of testing and were classed as healthy.

Anthropometrics

The standing height of the participants was measured with a portable Stadiometer (SECA, Switzerland) to the nearest 0.1 cm. A digital scale (SECA, Switzerland) was used to record body mass with participants wearing light indoor clothing. The body mass index (BMI) was calculated as body mass divided by height squared.

DEXA

Participants wore a medical gown and laid supine on the scanning bed. A total body DXA (Lunar Prodigy Advance, GE Healthcare, Chicago, USA) scan was performed to measure total body composition. Estimations of FFM and FM mass were obtained using Prodigy, Encore 2006 v10.50.086 software (GE Healthcare). Each total body scan took 295 s with an estimated skin entrance dose of 0.4 μGy (GE Healthcare, Lunar encore, Safety and Specification Manual). The system was calibrated with the same phantom at baseline and at 5 years follow up before each scan. All DXA analyses were completed by the same investigator. Typically, the estimates of lean mass by DXA software packages include connective tissue, non-mineral components of bone and non-adipose components of fat tissue alongside muscle mass. As the contribution of these factors is uncertain and possible changes of these components with aging unknown, we did not correct for these potential confounders.

Magnetic Resonance Imaging

Thigh volume was measured using a 0.25-T MRI scanner (G-Scan, Esaote, Genova, Italy). The participant was in a supine position in the scanner and multiple 3.1-mm thick serial transverse sections were acquired every 25 mm from the proximal to the distal heads of the femur of the dominant leg, using a turbo 3D58 T1-weight protocol (matrix 256 x 256, TR 40 ms, TE 16 ms). The cross-sectional area of the quadriceps

muscle and other thigh muscles (hamstrings, abductors and adductors) in each slice were determined using computing imaging software (OsiriX medical imaging software, OsiriX, Atlanta, USA). The cross-sectional area of the quadriceps muscle (CSA_{Quad}) was estimated using the maximal cross-sectional area from the serial transverse sections. Total cross-sectional area of the thigh musculature (CSA_{thigh}) was estimated from the maximal cross-sectional area using previously outlined methods (Morse et al., 2007a, McPhee et al., 2009).

Balance

Balance was determined as described previously (McPhee et al., 2013). Testing encompassed two-leg and one-leg trials, firstly with eyes open and then with eyes closed. Participants attempted to stand still for a maximum of 30 s or until one of the termination criteria was fulfilled. Participants removed shoes and had a visible marker placed on a wall 2 m in front of the participant to provide a fixed point during the eyes open trials. Two legged trials were completed with arms relaxed and feet together, participants were not permitted to use their arms to maintain balance. One-legged trials required the contra-lateral leg held to be held 5cm off the ground. All trails were repeated twice unless the participant failed to maintain balance for 30 s, in such cases a third trial was performed. Participants were encouraged to take some small steps between trials. Here we only report the time (in s) a person could stand on one leg with eyes open and eyes closed as a measure of balance.

Six-Minute Walk Distance

To assess the 6-minute walk distance two cones were placed 20 m apart. Participants were given the verbal instruction to “complete as many circuits as possible without running” and received verbal encouragement after each minute of the walk. The total

distance walked during the six-minute period was recorded (Enright, 2003). Heart rate was monitored throughout the test (Polar, USA) and the average heart rate during the final 3 minutes of the test was given as the steady state heart rate (S-shr). All participants completed the 6-minute walk without the use of a walking aid.

Timed Up-and-Go

The TUG test involved getting up from a standardised chair (no arm rests, seat 44 cm high) and to walk forward as quickly as they were able, without running, to a cone 3 m away and return to the initial sitting position. Participants were familiarised to the procedure prior to the execution of the real test. Upon the 'go' signal, participants rose from the chair and timing was concluded when seated again. The test was conducted three times for each participant, with a rest period of 1 min between trials, and the quickest of the three trials was recorded.

Cardiac Output Measures

Blood pressure and heart rate were taken from the left arm, using an upper arm blood pressure monitor (M2, Omron, Kyoto, Japan). From the diastolic and systolic blood pressures the mean arterial (MAP) and pulse pressure (PP) were calculated. The maximal heart rate (HR_{max}) was estimated as (Tanaka et al., 2001):

$$HR_{max} = 208 – (0.7 \times Age)$$

and (resting) stroke volume (SV) was calculated as (de Simone et al., 1999):

$$SV = PP \times (0.013 \times body\ mass – 0.007 \times age – 0.004 \times HR_{rest} + 1.307)$$

The SV and HR_{rest} allowed us to calculate (resting) cardiac output (CO) and peripheral resistance (R_{per}) (in $mmHg \cdot mL^{-1} \cdot min$):

$$R_{per} = MAP / CO$$

Maximal Cardiac ouput (CO_{max} in $L \cdot min \cdot^{-1} kg^{-1}$) was estimated as: HR_{max} * SV/BM.

Muscle Power

A maximal countermovement jump was performed on a force platform (Leonardo, Novotec Medical, Pforzheim, Germany) to measure the power of the leg extensor muscles. The participant was asked to perform the test three times (hands on the waist and no swing), with a 1-min rest between jumps. The best jump was selected for further analysis. The vertical component of the ground reaction force was used to calculate: jump height (m), maximal force (kN), maximum power of the concentric phase (Watts) and take-off velocity during the countermovement jump (V_{CMJ} in $m \cdot s^{-1}$) (Caserotti et al., 2001). Jump velocity at take-off was calculated as:

$$v = a \times t_f / 2$$

Where 'a' is the gravitational acceleration (9.81 m s^{-2}) and 't_f' the flight time of the jump. The flight time was the time from take-off until landing (the point which forces were registered on the platform again) (Degens et al., 2019).

Isometric maximal voluntary contraction torque

Isometric knee extensions were performed with the right leg on a custom-made isometric testing dynamometer (Designed by the Department on Physical and Medical Technology, VU University, Amsterdam, The Netherlands). Force signals were recorded via customised Labview (National Instruments Corporation, Texas, USA) and Matlab software (Matlab, the Mathwork Inc, S Natik, MA, USA). All procedures were explained to the participants, emphasising the requirement to stay relaxed and only to voluntary contract when instructed to do so. The participants were seated on

the dynamometer with a knee angle of 90° (full extension being 0°) and 85° hip flexion (supine being 0°). The lower leg of the participants was securely fastened to the force transducer, 2 cm above the ankle malleolus. The hip joint was firmly held in place via a strap. Prior to the measurements, the participants were familiarised to the knee extension exercise with three contractions at around 50% of maximal effort lasting 3 s each, followed by two further contractions at around 80% maximal effort lasting 3 s each. A two-minute rest was given prior to a MVC sustained for around 3 s. Two or more maximal contractions were performed until the two highest values were within 10%, with the highest value taken as MVC. Verbal encouragement and visual feedback were conveyed during the testing. This method was tested for reproducibility in a validation study (Appendix 1).

Statistics

Data was analysed using SPSS v22 (IBM, 2015). A repeated-measures two-way ANOVA with as within factor Time (baseline *vs.* follow up) and between factor Gender was used to examine differences over time and between genders. To determine relationships between the dependent variables (6MWD & TUG) and independent variables linear regression models were used. Data were expressed as mean ± standard deviation unless stated otherwise and differences were considered significant at $P<0.05$.

4.4 Results

Participant characteristics and muscle mass

Table 4.1 Men were heavier and taller than women (P<0.001). Both men and women became around 1 cm shorter over the 5-year period (P<0.001). There was a gender*time interaction for BMI (P=0.045) reflected by a decrease in BMI in women and an increase in men. Women had a lower FFM (P<0.001) and higher %FM (P=0.025) than men. Both genders lost FFM (P≤0.001), but there was no significant change in FM or %FM over the 5-year period. Appendicular lean mass, sarcopenia index, CSA_{Quad} and CSA_{thigh} all decreased over time (P<0.001), with men losing more CSA_{Quad} than women (time * gender interaction, P=0.029).

Table 4.1. *Participant characteristics*

	Women (n=17)			Men (n=17)			Effects		
	Baseline	Follow-up	% change	Baseline	Follow-up	% change	Time	Gender	Gender*Time
Age (years)	71.1±2.8	75.9±2.6		71.3±4.1	75.9±4.4		P=0.000	P=0.924	P=0.562
Body mass (kg)	65.8±10.5	64.2±11.3	-2.74	84.2±15.5	84.4±15.4	0.24	P=0.174	P=0.000	P=0.093
Height (m)	1.61±0.07	1.59±0.06	-1.24	1.75±0.07	1.74±0.07	-0.57	P=0.000	P=0.000	P=0.774
BMI ($kg \cdot m^{-2}$)	25.8±5.34	25.5±5.56	-1.16	27.6±4.47	28.0±4.20	1.45	P=0.905	P=0.186	P=0.045
FFM (kg)	39.0±3.04	37.9±3.20	-2.82	56.2±7.37	55.2±6.91	-1.78	P=0.002	P=0.000	P=0.861
FM (kg)	24.5±9.60	24.1±10.5	-1.63	24.7±10.5	25.8±10.5	4.45	P=0.376	P=0.798	P=0.107
FM (%)	37.4±9.34	37.4±10.0	0.00	29.4±8.75	30.8±8.51	4.76	P=0.163	P=0.025	P=0.163
ALM (kg)	17.4±1.80	16.8±1.76	-3.45	26.2±3.60	25.0±3.29	-4.58	P=0.000	P=0.000	P=0.063
Sarcopenia index ($kg \cdot m^{-2}$)	6.70±0.46	6.56±0.41	-2.09	8.58±0.88	8.28±0.68	-3.50	P=0.001	P=0.000	P=0.174
CSA_{Quad} (cm^2)	44.2±6.27	42.8±5.94	-3.17	64.6±9.99	58.7±7.95	-9.13	P=0.000	P=0.000	P=0.029
CSA_{thigh} (cm^2)	94.0±18.8	89.1±17.2	-5.21	125±33.3	118±32.6	-5.60	P=0.000	P=0.005	P=0.294

BMI: Body Mass Index; FFM: Fat Free Mass; FM: Fat Mass; ALM: Appendicular Lean Mass; CSA_{Quad}: cross-sectional area quadriceps muscle; CSA_{thigh}: cross-sectional area thigh

Balance, TUG and 6MWD

Both men and women had a decrease in balance time with eyes open (Table 4.2; P<0.001) and eyes closed (Table 4.2; P<0.05). Women needed more time to complete the TUG than men (P=0.003), but covered a similar 6MWD. For both men and women, the performance in the TUG and 6MWD test decreased over the 5-year period (Table 4.2; P<0.001).

Table 4.2. *Measures of mobility*

	Women (n=17)			Men (n=17)			Effects		
	Baseline	Follow-up	% change	Baseline	Follow-up	% change	Time	Gender	Gender*Time
1_{leg}EO (s)	25.3±8.03	17.8±10.5	-29.64	24.6±9.20	15.2±10.0	-38.21	P=0.000	P=0.610	P=0.554
1_{leg}EC (s)	6.53±5.34	3.31±1.97	-49.31	5.95±5.21	3.93±1.92	-33.95	P=0.015	P=0.984	P=0.562
TUG (s)	5.68±0.59	7.07±1.21	24.47	5.11±0.59	6.01±0.85	17.61	P=0.000	P=0.003	P=0.150
6MWD (m)	533±83	494±69	-7.32	568±59	514±59	-9.51	P=0.000	P=0.226	P=0.434
S-Shr	119±12	113±11	-5.04	111±17	104±15	-6.31	P=0.000	P=0.106	P=0.759
6MWD % HR_{max}	75.7±10	73.2±7.3	-4.4	69.8±11.1	66.6±9.3	-4.58	P=0.001	P=0.086	P=0.665

1_{leg}EO: one leg balance eyes open; 1_{leg}EC: one leg balance eyes closed; TUG: timed up and go; 6MWD: six-minute walk distance; S-Shr; six-minute walk steady state heart rate; 6MWD % HR_{max}: six-minute walk percentage of maximum predicted heart rate

Cardiovascular parameters and Muscle function

There was an increase in resting HR (Table 4.3; P=0.006), and decreases in BP_{dia} (Table 4.3; P=0.027) and calculated maximal cardiac output (Table 4.3; P=0.004). Other cardiovascular parameters did not change significantly over the 5-year period.

Standing jump power, V_{CMJ}, standing jump force and MVC of knee extensor muscles were all higher in men than in women (Table 4.4; P≤0.001), with both genders showing a decrease in power, V_{CMJ} and MVC over the 5-year period (Table 4.4; P≤0.005).

Table 4.3. *Cardiovascular parameters*

	Women (n=17)			Men (n=17)			Effects		
	Baseline	Follow-up	% change	Baseline	Follow-up	% change	Time	Gender	Gender*Time
HR_{rest} (bpm)	68.6±7.19	73.6±8.56	7.29	64.6±8.80	67.5±7.71	4.49	P=0.006	P=0.067	P=0.551
BP Dia (mmHg)	80.7±11.3	74.0±8.73	-8.30	83.0±9.6	82.5±7.8	-0.60	P=0.027	P=0.086	P=0.056
BP Sys (mmHg)	136±18.1	129±21.0	-5.15	135±17.5	135.±16.2	0.00	P=0.130	P=0.667	P=0.124
MAP (mmHg)	93.4±27.0	93.3±11.8	-0.11	93.8±26.6	94.1±25.8	0.32	P=0.992	P=0.920	P=0.974
Pulse Pressure (mmHg	55.6±14.5	55.0±17.1	-1.08	52.3±12.1	52.9±13.1	1.15	P=0.995	P=0.574	P=0.741
Stroke Volume (mL)	77.7±24.4	72.7±25.3	-6.44	86.7±27.0	85.5±26.1	-1.38	P=0.312	P=0.225	P=0.518
Cardiac Output (mL/min)	5.29±1.67	5.38±2.14	1.70	5.54±1.79	5.72±1.63	3.25	P=0.576	P=0.637	P=0.842
R_{per} (mmHg-min-mL^{-1})	20.1±5.16	19.1±5.62	-4.98	19.3±4.47	18.8±5.28	-2.69	P=0.364	P=0.752	P=0.792
CO_{max} ($mL \cdot min^{-1} \cdot kg^{-1}$)	173±60.3	149±52.9	-13.8	194±64.1	176±55.1	-9.30	P=0.004	P=0.244	P=0.643

HR_{res}: Heart rate at rest; BPdia: diastolic blood pressure; BPsys: systolic blood pressure; MAP: mean arterial pressure R_{per}: peripheral resistance CO_{max}: cardiac output maximum

Table 4.4. *Muscle function*

	Women (n=17)			Men (n=17)			Effects		
	Baseline	Follow-up	%change	Baseline	Follow-up	% change	Time	Gender	Gender*Time
Standing Jump Power ($W \cdot kg^{-1}$)	23.7±5.9	21.6±5.0	-8.86	29.9±4.7	26.7±4.8	-10.70	P=0.000	P=0.003	P=0.277
V_{CMJ} ($m \cdot s^{-1}$)	1.94±0.19	1.81±0.18	-6.70	2.22±0.19	2.18±0.26	-1.80	P=0.009	P=0.000	P=0.171
Standing Jump Force (kN)	1.32±0.32	1.38±0.26	4.55	1.76±0.40	1.82±0.30	3.41	P=0.166	P=0.000	P=0.978
Knee Extensor MVC (N)	417±72	366±75	-12.23	595±83	518±108	-12.94	P=0.000	P=0.000	P=0.344

V_{CMJ} : take-off velocity during the countermovement jump; MVC: Maximal voluntary torque

Correlations of mobility with cardiac output and muscle function

In follow up, the S-shr during the 6MWD and the distance covered in the test were lower than at baseline (Table 4.2; P≤0.001). The $\%HR_{max}$ during the 6MWD was also reduced (Table 4.2; P≤0.001), suggesting that the reduction in maximal heart rate is not the sole explanation for the reduction in 6MWD performance.

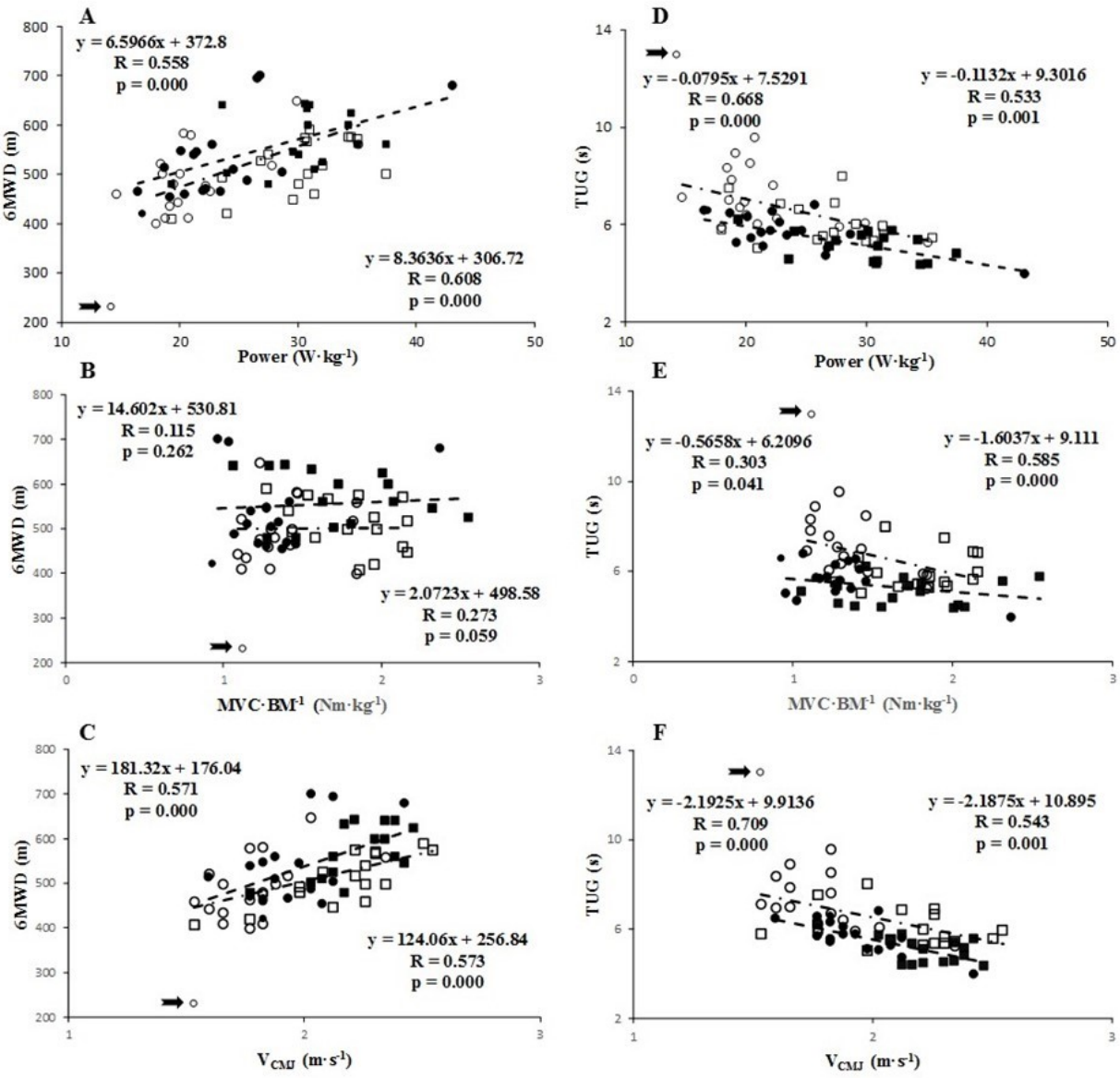

Figure 4.1. The relationship between **A-C)** 6-minute walking distance (6MWD; m) and **D,F)** timed up-and-go (TUG; s), with **A,D)** power (W/kg), **B,E)** maximum voluntary torque / body mass (MVC-BM^{-1}; Nm·kg^{-1}), **C,F)** take-off velocity during the countermovement jump (V_{CMJ}; m·s^{-1}). ■: men and ●: women at baseline, and □: men and ○: women at follow up. ---: regression line at baseline; -·-: regression line at follow-up. Regression equation left at baseline, right at follow-up. Arrow indicates woman with poor performance in follow up.

Figure 4.1 showed only at follow up did balance, in terms of the time standing on one leg with eyes closed, correlate with the 6MWD (R=0.47; P=0.002) and TUG (R=0.39; P=0.01). Both at baseline (R=0.56; P<0.001) and follow up (R=0.61; P<0.001) the 6MWD correlated with power (Fig. 4.1A). The performance in the 6MWD did not correlate significantly with $MVC \cdot BM^{-1}$ (Fig. 4.1B), but did correlate with V_{CMJ} (Fig. 4.1C) at baseline (R=0.57; P<0.001) and follow up (R=0.57; P<0.001).

Figure 4.1D shows a positive correlation (indicated by the negative slope) between power and performance of TUG at baseline (R=0.67; P<0.001) and follow up (R=0.53; P<0.001). The $MVC \cdot BM^{-1}$ correlated to TUG at both baseline (R=0.30 P=0.041) and follow-up (R=0.59 P=0.000) (Fig. 4.1E). The performance of the TUG also correlated positively with V_{CMJ} (Fig. 4.1F) at baseline (R=0.71; P<0.001) and follow-up (R=0.543; P<0.005).

Velocity and body mass

V_{CMJ} was inversely correlated with the $BM \cdot MVC^{-1}$ ratio both at baseline and follow-up (both R>0.54; P<0.001; Fig. 4.2). The % change in power was not significantly related to baseline power (Data not shown; R=0.196 P=0.133).

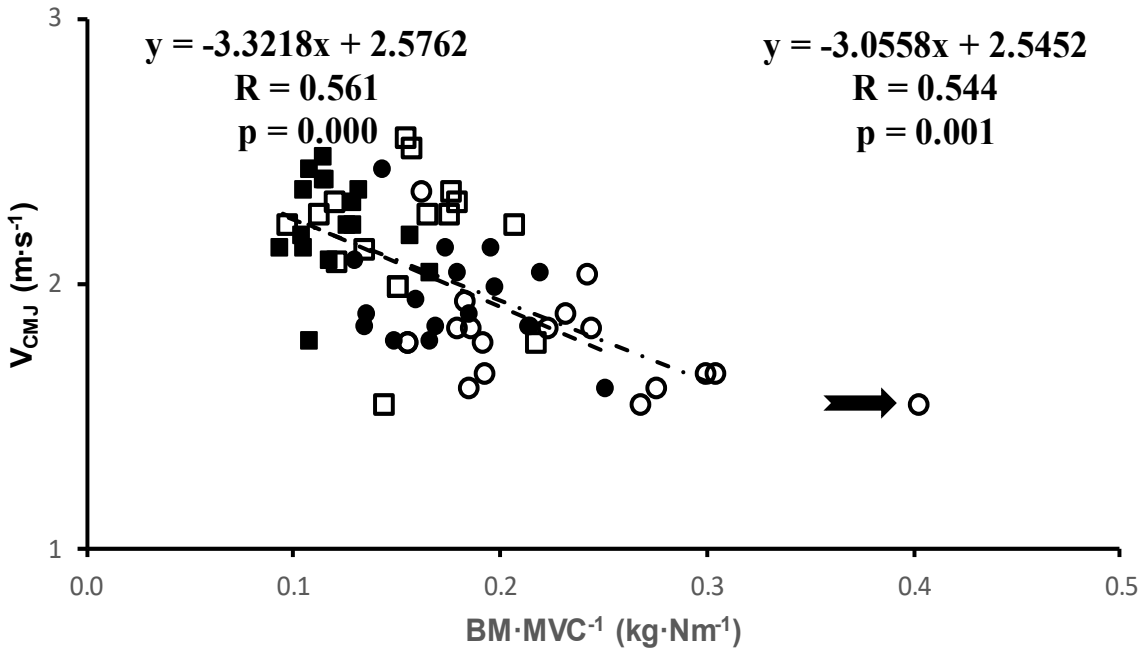

Figure 4.2. Body mass / MVC (kg·Nm^{-1}) with velocity (m·s^{-1}). ■: men and ●: women at baseline, and □: men and ○: women at follow up. ---: regression line at baseline; -·-: regression line at follow-up. Regression equation left at baseline, right at follow-up. Arrow indicates woman with poor performance in follow up. One person performs poorly in both the 6MWD and TUG during follow up (indicated with an arrow in all figures)

Note that one person performed poorly in both the 6MWD and TUG during follow up (indicated with an arrow in all figures). This person had an S-shr in the normal range during the 6MWD, but low power. This low power was attributable to a reduction in the force generating capacity and not so much slowing of the muscle as the lower V_{CMJ} was as expected from the BM·MVC^{-1} for this person.

4.5 Discussion

The novel observation in this longitudinal study was that healthy septuagenarians suffer from a significant decline in muscle mass/function and mobility over a 5-year period, irrespective of gender. The annual decline was larger than that observed in a previous cross-sectional study comparing 23- and 72-year-old people (Maden-Wilkinson et al., 2015), suggesting an accelerated age-related decline beyond the age of 70. Muscle power, determined with a countermovement jump, correlated most with performance in the 6-minute walk and timed up-and-go tests, at both baseline and follow-up, while balance was associated with performance at follow-up only. The loss of power in the septuagenarians was primarily due to a reduction in force generating capacity, rather than a further slowing of the muscle. These results suggest that muscle power is a key determinant of physical function during relatively long- and short-duration physical function tasks and that with advancing older age balance is of increasing importance for physical function.

Decline in muscle function and physical function

The main defining features of sarcopenia are low muscle mass, weakness and reduced physical function (Fried et al., 2001, Cruz-Jentoft et al., 2019). The ALM/h2 were at baseline and follow-up above the sarcopenia cut-offs for men (7.26 $kg \cdot m^{-2}$) and women (5.5 $kg \cdot m^{-2}$) (Cruz-Jentoft et al., 2019), suggesting that our participants were not sarcopenic according to the accepted cut-points. While an increase in TUG time is associated with an amplified risk of a falling, decline in physical function and an increase in frailty index (Beauchet et al., 2011, Viccaro et al., 2011, Kojima et al., 2015), the TUG time at follow up was still (except the woman excluded from analysis) well below the 12-s cut-off point for normal mobility (Bischoff et al., 2003), suggesting they were neither physically frail. Similarly, the 6MWD is commonly used to assess

functional capacity (Enright and Sherrill, 1998, Troosters et al., 1999). As with the TUG test, even though performance in the 6MWD decreased over the 5-year period, all participants (again except the excluded woman) covered ≥ 400 m at follow up, considered the cut-off for mobility limitations (Abellan van Kan et al., 2011). These observations thus indicate that the participants in our study were not sarcopenic according to the cut-offs for skeletal muscle mass, nor (except one older woman at follow-up) physically frail, but rather a population of healthy ageing.

Accelerated decline

The annual reduction in 6MWD and TUG performance was larger in the present longitudinal study (0.9 & 4.2%, respectively) than that calculated from a previous cross-sectional study (0.4 & 0.7%) (Maden-Wilkinson et al., 2015). This and the annual decline in jumping power of 0.8% from a previous study (Maden-Wilkinson et al., 2015) compared to 2.0% in the present longitudinal study, suggest an accelerated decline in functional capacity beyond the age of 70 years. These calculations assume that human peak performance occurs early in the third decade, something that has been observed in master athletes of several disciplines (Ganse et al., 2018, Berthelot et al., 2012). Therefore, our findings suggest an accelerated decline in muscle power in the 8th decade of life cannot solely be due to decreased physical activity levels, since similar declines are evident even in athletic populations (McPhee et al., 2018, Frontera et al., 2000a, Frontera et al., 2008, Lazarus and Harridge, 2017, Degens, 2012). If that decline continues to progress at the same, or even an accelerated, rate it will ultimately result in a transition from an independent to a dependent lifestyle, even in our population who were non-sarcopenic and free from physical limitations in daily life. It is therefore important to uncover the ageing-related changes that elicit these accrued deficits in functional capacity.

Contribution of aerobic component and balance to the decline in 6MWD and TUG

Part of the poorer performance in the TUG and 6MWD during follow-up was associated with an impaired balance, something also reported previously in people > 70 years (Chen and Chou, 2017). Even before the age of 70, a significant reduction in balance occurs (Onambele et al., 2006). However, the absence of significant correlations between balance, assessed as the duration one could stand on one leg with eyes closed, with 6MWD and TUG performance at baseline suggests that only after the balance impairment exceeds a certain threshold it becomes a limiting factor for daily life performance.

The 6MWD clearly requires an aerobic component and it is related to the maximal oxygen consumption of healthy young-adults and older people (Manttari et al., 2018). The heart rate measured during the 6MWD was decreased over the 5-year period both as absolute values and when expressed relative to the estimated age-predicted maximum. This reflects both a decrease in cardiovascular function and a decrease in the relative effort that the older adults were willing to put in when walking. It is not clear why less effort would be applied during the walk and our study methodology cannot reveal the reasons in any detail. However, it may be related to concerns over balance and the risk of falling. This supports previous studies of people aged > 70 years (Chen and Chou, 2017) and suggests that deterioration of balance makes an increasing contribution to physical functional declines with advancing age, either directly as adjustments are made with each step to control posture, or indirectly through more caution due to fear of falling.

Contribution of muscle function to the decline in 6MWD and TUG

Previous studies have shown that the performance in the 6MWD and TUG tests are related to muscle mass and function (Song and Geyer, 2018, Maden-Wilkinson et al., 2015, Bijlsma et al., 2014, Janssen et al., 2002). However, the relationship between functional limitation and muscle mass in older people is weak (Lauretani et al., 2003) or even absent (Maden-Wilkinson et al., 2015) and we show that power is more important. In the previous chapter we found that the ageing-related loss of muscle strength during early ageing is due to both a loss of muscle mass and quality, in terms of force generating capacity per unit muscle cross-sectional area, while in the 8th decade of life it is primarily due to a loss of muscle mass (McPhee et al., 2018). It is thus possible that in the oldest-old a low muscle mass becomes an increasingly important contributor to reduced functional capacity. Yet, we found neither a significant relationship between muscle mass and functional capacity at baseline nor at 5-year follow up. This confirms the increasing notion that not so much muscle mass, but rather muscle functional capacity is relevant as a determinant of the ability to perform daily life activities in the older population, even in people in the 8th decade of life. Given that power is the product of force and velocity one can understand that muscle power has been reported to better correlate with functional capacity than maximal voluntary isometric force in the older population (Reid and Fielding, 2012, Maden-Wilkinson et al., 2015). This was confirmed in our longitudinal cohort at both baseline and 5-year follow up.

It has previously been shown that the difference in jump power between young and older subjects is in part due a reduction in force generating capacity and in part due to slower contractile properties of the muscle. While power increased with force, for a given jump force, power was greater in the young subjects (Maden-Wilkinson et al.,

2015). This was attributed to a reduction in the intrinsic speed of shortening of the older muscle, probably a consequence of an ageing-related fast-to-slow transition in fibre type composition (Larsson and Ansved, 1995), preferential atrophy of fast fibres (Barnouin et al., 2017b) and/or a slowing of type I and type IIa muscle fibres (Larsson et al., 1997, Degens et al., 1998).

However, when we looked further into this apparent slowing over the 5-year period, it appeared that there was no significant change in the body mass:maximal force ratio (Fig. 4.2). This means that both at baseline and follow-up, at a given 'body mass:maximal force ratio' the shortening velocity during the countermovement jump is the same. Given that body mass was not significantly changed over the 5-year period, but force was reduced by about 12%, the slower take-off velocity in the countermovement jump must have been the consequence of loss of force generating capacity. Thus, the actual culprit behind this apparent slowing of the muscle is that they are working at a higher relative load than 5 years prior, and therefore contracting more slowly according to the force-velocity relationship. Thus, while intrinsic slowing of the contractile properties was the main determinant of the ageing-related reduction in jump velocity between 23 to 72 years of age (Maden-Wilkinson et al., 2015), it is the loss of force that causes a further decline in power in the ageing septuagenarian. It should be noted that with ageing jumping kinematics are influenced, older individuals maximal hip, knee and ankle angular velocities are significantly decreased compared to young, with jump performance limited by reduced angular range of motion and loss of sequential pattern of joint coordination (Haguenauer et al., 2005). It remains to be seen if such changes continue to influence jumping kinematics during later ageing (71-76 years).

An interesting pattern therefore arises that during early ageing (23-72 years) particularly intrinsic slowing contributes to the loss of power (Maden-Wilkinson et al., 2015) and that weakening is the result of loss of muscle mass and quality (McPhee et al., 2018). On the other hand, during later ageing (71-76 years) loss of force, due to a loss of muscle mass and reduced voluntary activation (McPhee et al., 2018), but not further loss of muscle quality, is the primary contributor to the ageing-related loss of power.

As previously mentioned this loss of power may eventually lead to a transition from an independent to a dependent life style. It has been shown; however, that muscle strength is positively related to physical activity levels in the older person (Latorre-Roman et al., 2016). An increase in physical activity levels may thus reverse the loss of strength in the older person and improve functional performance. Indeed, resistance exercise is a potent means to improve functional performance, even in the oldest old (Fiatarone et al., 1990).

Conclusion

In conclusion, the ageing-related reduction in functional capacity over a 5-year period in healthy septuagenarians was to some extent attributable to a reduction in maximal heart rate and balance. However, a larger proportion of the decline in performance during a 6-min walk and timed up-and-go tests was explicable by a decline in muscle power. In contrast to the intrinsic slowing of the muscle between 23 and 72 years of age (Maden-Wilkinson et al., 2015), the further decrement in power and performance of the functional tests between 71 and 76 years of age was primarily attributable to loss of strength. This suggest that the process of muscle ageing may change from

decrements in both muscle mass and quality during early ageing to principally a reduction of muscle mass during later stages of ageing.

Chapter 5

General Discussion

5.1 Aims and Objectives

The overall aim of this thesis was to 1) assess longitudinal changes in muscle size and function over a 5-year period and 2) how these changes affected the ability to perform activities of daily life, in physically-independent older individuals. To achieve this the study had the following objectives:

1) To assess the reliability of DXA measurements to assess longitudinal changes in muscle mass in older populations.

2) To uncover the gross functional basis of the age-related changes in mobility seen in older individuals.

3) To describe the influence of fibre atrophy, fibre loss, in situ specific force, and voluntary activation to muscle weakness seen with ageing.

5.2 Main observations

Each of these Objectives was addressed in the studies described in Chapters 2-4, respectively. The detailed longitudinal examination of this population was a key part of the studies, and is unique where most studies on muscle ageing are cross-sectional. In addition, our study population was more homogenous than that in larger epidemiological studies of ageing which include a wide-ranging participant base, with many disabilities and co-morbidities. The participants in this study exhibited healthful ageing, being community dwelling, socially active, with no known musculoskeletal or cardiovascular diseases (McPhee et al., 2013).

Ageing-related changes in muscle mass

While the measurement of total volume by MRI is considered the gold standard, it is time consuming and costly. In Chapter 2, we showed that a single MRI scan at 60% of femur length could be used to accurately calculate muscle volume and changes therein in septuagenarians, as was previously shown in young men (Morse et al., 2007a, Maden-Wilkinson et al., 2014). Reducing the time and cost required to perform such analysis, making MRI a more viable modality for larger cohort studies, allowing much greater detail to be described.

Even so, MRI is not widely available or easy to use, while DXA is more widely available and commonly used in large cohort studies to measure muscle volume (Ellis, 2000, Visser et al., 2003, Goodpaster et al., 2006, Zhong et al., 2012, Santanasto et al., 2017). While in Chapter 2 it was found that in both men and women DXA muscle volume measurements correlated well with MRI, DXA displayed a positive intercept with MRI and the slope of the regression line was greater than 1. As a consequence DXA consistently overestimated muscle volume, as seen in our previous work (Maden-

Wilkinson et al., 2013b). However, the discrepancy over the 5-year time scale between DXA and MRI was relatively small, signifying that DXA is a viable and reliable method for tracking muscle mass longitudinal in older people. Extending the ability to identify changes in mass which may lead on to functional impair within older populations.

Chapter 2 described muscle mass decreases of 5% over the 5-year period in septuagenarians, suggesting an accelerated decline in these older individuals when compared to the 25% change described between individuals in their twenties and seventies (Maden-Wilkinson et al., 2014). There was no differential rate of loss between quadriceps muscles and other muscles in the thigh, in contrast to what was reported in a previous cross-sectional study (Maden-Wilkinson et al., 2013b). We demonstrated that the relative rate of muscle mass loss with ageing was not related to baseline muscle mass, thus indicating the benefit of having a larger muscle mass to start with, as it will delay crossing the disability threshold of muscle mass until later in life (Degens, 2018, Degens and McPhee, 2013). While in previous studies the rate of muscle wasting was positively related to body fat percentage in women (Tomlinson et al., 2014) we observed this relationship in men only. A possible mechanism is that fat tissue may lead to muscle wasting due the release by fat of inflammatory cytokines resulting in chronic low-grade inflammation (Degens, 2010), which may impair the rate of nutrient stimulated muscle protein synthesis rates (Smeuninx et al., 2017). Though the influence of circulated inflammatory cytokines linked with obesity may have much wider influence on skeletal muscle size, architecture and strength (Erskine et al., 2017). The contractile component of the total volume of the whole muscle is decreased with intramuscular fat infiltration seen with increased body fatness (Rahemi et al., 2015). This in turn will influence muscle quality and intermuscular adipose tissue may be a predictor of muscle function in older populations (Beavers et al., 2013).

Ageing-related changes in muscle function

The loss of muscle mass seen with ageing is inevitably associated with a loss of muscle strength, leading to deficits in functional performance. Having described changes in muscle mass longitudinally in Chapter 2, the contribution of fibre atrophy, fibre loss and reduction in voluntary activation to the muscle weakness was investigated in Chapter 3. It was observed that patella tendon specific force was 17% lower in old compared to young, but during a 5-year follow-up there was no further significant decrement in specific force in the older population.

Using measurements of muscle volume and muscle architecture, the PCSA was calculated. In Chapter 3 it was estimated that 54% of the difference in PCSA between young and old was due to fibre atrophy, with fibre losses accounting for the remaining 46%. The number of fibres lost was an estimated 200,000, and must have been preferential loss of type I fibres, due to the lack change seen in the relative area occupied in the muscle by type I and type II fibres between young and old in the face of preferential type II fibre atrophy. Others (Nilwik et al. (2013) did not find evidence for fibre loss, and in their study the age-related atrophy could be entirely explained by muscle fibre atrophy. A possible explanation of this discrepancy is that their subjects had much larger muscles than our subjects, and they were non-sarcopenic in contrast to our subjects. Whatever the explanation for this discrepancy, the loss of fibres in our study is thought to be linked to the loss of motor units seen with ageing (30-50% fewer) (Piasecki et al., 2016b, Tomlinson and Irving, 1977). Overall, the data suggested that loss of muscle mass was the main cause of the age-related muscle weakness, but there is also a significant contribution of a loss of muscle quality, defined here as specific force (force per PCSA), as also found by others (Morse et al., 2005a).

In a murine model, it was shown that part of the lower specific force in old animals was due to accumulation of connective tissue (Ballak et al., 2014). In our study this only accounted for 3% of the variation seen. In addition, we reported a preferential atrophy of type II fibres that may have a 40% higher specific tension than type I fibres (Bottinelli et al., 1996), though others report no significant difference in specific force between fibre types (Ottenheijm et al., 2005, Degens et al., 2009b). Nevertheless, even if we assume such a difference in specific tension, the change in areal fibre type proportion is too small to have a significant impact. It is possible that the lower specific force of the muscle is primarily due to lower specific tension of individual muscle fibres, which has been reported to be 16% lower in old vs young (Brocca et al., 2017, D'Antona et al., 2003, Larsson et al., 1997), matching with the data reported here.

Ageing-related changes in mobility related to changes in muscle function

Sarcopenia is considered an important factor in frailty in older people (McPhee et al., 2018, Maden-Wilkinson et al., 2015, McPhee et al., 2016). The aim of Chapter 4 was to assess the relationship between changes in muscle function with changes in performance of daily life activities in a 5-year longitudinal study of septuagenarians.

A significant decline was shown in all measurements of physical function and muscle function, with the annual decline in muscle force generating capacity being much larger than that shown in our previous cross-sectional study between young and older individuals (Maden-Wilkinson et al., 2015). More specifically, the 6MWD and TUG showed an annual decline of 0.9% and 4.2% over the 5-year period, in comparison to a 0.4 and 0.7%, respectively, in the preceding years. The annual decline in jumping power was 2.0% compared to the 0.8% suggesting an accelerated decline in muscle power in the oldest old (Degens and Korhonen, 2012).

Part of the reduced performance in the 6MWD may be due to a decrease in maximal heart rate over the 5-year period, as it has been suggested that the walking speed occurs at around 83% of maximal heart rate (Manttari et al., 2018). In Chapter 4 it was found that older people walked at an even lower percentage of their maximal heart rate, suggesting that something else than cardiovascular limitations may diminish walking speed, such as limited range of motion and pain.

Besides the above factors, decrements in muscle mass and function may contribute to a reduced performance in daily life activities (McPhee et al., 2016, Reid and Fielding, 2012, Larsson et al., 2019). In contrast to previous studies (Bijlsma et al., 2014, Janssen et al., 2002), we found no relationship between muscle mass and functional capacity at both baseline and follow-up. In fact, muscle functional capacity is more important in this older population to perform daily life activities than muscle mass *per se,* with power and MVC reported to correlate better in older people (Reid and Fielding, 2012, Maden-Wilkinson et al., 2015), something we also observed in our work.

Power appeared to be the strongest predictor of 6MWD and TUG performance. Therefore, in Chapter 4 the influence of the two components of muscle power, force and velocity, for performance were studied. It was found that the velocity of take-off during a counter movement jump was correlated with functional performance at both baseline and follow-up, and it was concluded that this was due to both a loss of force and an intrinsic slowing of the contractile properties, as suggested previously (Maden-Wilkinson et al., 2015, Macaluso and De Vito, 2004). However, in the 5-year follow-up study, no significant changes in the body mass:maximal force ratio (Fig. 4.2) were found, suggesting that the slower velocity at take-off was due to either an increased body mass and/or weakening of the muscle, but no further slowing of the muscle. As

there was a 12% reduction in force and no significant change in body mass over the 5-years, the lower shortening velocity was a consequence of decreased force generating capacity. The apparent slowing of the muscle is thus due to the muscles working a relatively higher load (due to the loss muscle strength), leading to slower contraction according to the force-velocity relationship. The findings in chapter 3 and 4 allow more targeted exercise interventions in older populations, to help delay the onset of mobility issues and transition to a dependent lifestyle.

5.3 Limitations and directions for future research

This thesis showed significant loss of muscle mass and function, and functional capacity in septuagenarians in as short a period as 5 years. However, a number of fundamental questions are still unresolved. This work suggested accelerated ageing when individuals reach their eighth decade and in line with previous literature (Mitchell et al., 2012, Ganse et al., 2018, Degens and Korhonen, 2012). However, it needs to be confirmed through further studies in changes occurring from the fifth decade, characterising middle age and more work in those progressing into and past their eighth decade to provide insight into when this accelerated period of ageing begins or if it is present at all. All participants within the study were living independently and in relatively good health, and therefore the model of ageing described here may not be representative for wider population. We must consider that even though the model of ideal ageing may be linear, the majority of individuals will not follow a linear path. Life events such as illness or surgery are likely to have key impacts into how people age and changes in musculature and it is important that these changes are characterised alongside looking at frail or more severely sarcopenic individuals. It should also be noted that investigation of habitual physical activity was not conducted; therefore, it cannot be assumed that the loss of muscle mass and function seen within this work

were solely down to the normal ageing process, as they may have been changes in habitual physical activity that either accelerated or blunted these processes. It has been shown that the maintenance of physical activity is key in older individuals and that as little as two weeks reduced step count contributed to reductions of muscle mass and perturbations in other key process key in muscle maintenance (Breen et al., 2013). Such individuals may suffer from varying ailments that may accelerate muscle wasting and hence the rate of ageing. Such people, and even healthy older people as in our study, may improve their muscle function and quality of life by improving muscle function by e.g. aerobic & power training. Indeed, the efficacy of resistance training programmes to improve muscle mass and strength has been shown in older people, and even those with cancer, joint replacements etc. (Reeves et al., 2004b, Nilwik et al., 2013, Doherty, 2003a). However, these benefits do not stop the loss of function with ageing, with master athlete's still experiencing loss in endurance, strength and power. The maintenance of physical activity is also not enough to maintain muscle mass with ageing , with only an increased load of physical activity able to attenuate these changes over and above 'leisure time' activity (Mitchell et al., 2003). Resistance training is thought to lead to a decrease in catabolic and increase in anabolic pathways (Ribeiro et al., 2017). Increasing protein synthesis, tempering anabolic resistance (Schulte and Yarasheski, 2001), as well eliciting changes in the neuromuscular system (Taaffe et al., 1999), with these factors all lessening the impact of ageing on muscle quality and consequently mobility related issues. It is also important to consider the benefits of aerobic exercise on ageing muscle, alongside the cardiovascular conditioning it provides. Cross sectional area of older human muscle fibres and hypertrophy have been reported through the administration of aerobic exercise (Konopka et al., 2013). These effects are principally through an increase in

mitochondrial biogenesis, with long term aerobic exercise programmes decreasing ROS production in older people, alongside the benefit of reduced abdominal fat (Konopka et al., 2013, Short et al., 2003). To enhance the benefits of such programmes nutrition also needs to be considered in light of the anabolic resistance reported in old people (Rennie, 2009, Breen and Phillips, 2013). This may include increased protein intake and the supplementation of omega-3 fatty acids that further improve muscle mass and decrease fat mass (Park et al., 2018, Smith et al., 2011). It has been shown that a low protein diet can be detrimental and is linked with frailty in older adults (Coelho-Júnior et al., 2018). Increasing protein intake in older people can overcome anabolic resistance in older people, therefore increasing protein synthesis and muscle mass (Moore et al., 2015). Essential amino acid (EAA) profile should also be considered, as without complete EEA profile maximal protein synthesis cannot be achieved, with leucine being the key regulator of protein synthesis from ingested proteins (Volpi et al., 2003, Devries et al., 2018). All of which highlight the importance of regular physical exercise, healthy diet and low body fat in old age, to maintain muscle mass and function (McPhee et al., 2016, Mithal et al., 2013).

Although it was found that DXA can be used to assess the changes in body mass in an older populations, it consistently overestimates the actual muscle mass in comparison to the gold standard, MRI (Abe et al., 2015, Loenneke et al., 2016). This then will result in an underestimation of the specific force of muscle, which should be considered when comparisons are made with other values of specific force in the literature. The cause of this overestimate is as yet unknown.

The use of a single measure of mobility has limited value to identify those who have sarcopenia (Looijaard et al., 2018). Indeed, the most widely used criteria to define sarcopenia include a battery of measurements, such as functional performance,

muscle mass and muscle strength (Fried et al., 2001, Cruz-Jentoft et al., 2019). Also in this study more than one functional measure was used (6MWD and TUG) and related to muscle function. The strength of our work is that we were able to disentangle the contributions of force generating capacity and intrinsic slowing of the muscle to the age-related reduction in power, something rarely considered.

Voluntary activation measurement using twitch interpolation has been shown to have good intra-rater reliability (correlation coefficients > 0.80), though we should be aware that methodological flaws may cause issues with this measurement, such as resting twitch, stimulation location, inadvertent stimulation of antagonists and joint angle (Nuzzo et al., 2018).

5.4 Conclusion

The thesis's overall aim was to characterise the relationship between loss of mass and function with functional performance measures in UK-based septuagenarian men and women during a 5-year longitudinal study. It was found that DXA showed a similar percentage atrophy as MRI, demonstrating that DXA can be used to assess longitudinal changes in muscle mass in older people. The rate of muscle wasting was independent of baseline muscle mass in both sexes, a higher baseline body fatness led to a greater rate of muscle wasting in men. The annual percentage decline in muscle mass during the 5-year period was larger than that seen between people in their twenties and seventies (Maden-Wilkinson et al., 2015).

Significant decrements in mobility performance were reported in both genders that was to some extent attributable to an age-related decline in maximal heart rate and balance. The most significant contributor to the decreased performance in the 6-min walk and timed up-and-go tests was the age-related decline in muscle power. It

appeared that this loss of power in the 5-year follow-up was primarily due to a reduction in MVC rather than intrinsic slowing of the muscle that seemed to play a significant role during early ageing (Maden-Wilkinson et al., 2015).

The loss of force was a consequence of both a reduction in muscle mass and muscle quality (specific force; force per muscle cross-sectional area), and a minor contribution of a reduced ability to fully activate the muscle. This loss of muscle mass was the result of preferential type II atrophy and loss of fibres. The findings within this thesis have implications for the design of interventions to mitigate these age-related changes even in a septuagenarian population in relatively good health, delaying the transition from independent living to dependent living.

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Appendix 1

Reproducibility of contractile properties of the human quadriceps muscle in healthy young subjects

Introduction

The use of electrical stimulation techniques to assess human muscle contractile properties has been well established (Gerrits et al., 2001, Chan et al., 1999, Hunter et al., 1999, Degens et al., 2005, Morse et al., 2007b, Wust et al., 2008). Electrical stimulation allows the determination of muscle contractile indices such as force frequency relationship, fatigue index, maximum rate of contraction and maximum rate of relaxation, while avoiding possible motivational bias. The contractile properties determined in this way appear to correlate with a variety molecular or histochemical muscle features (Harridge et al., 1998).

When performing repeated measures it is important to know how reproducible the measurements are. This is particularly important when one wants to know the impact of an intervention on the contractile properties of the muscle, especially when one considers muscle may adapt quickly to changes in functional demands (Baar and Hargreaves, 2011, Koopman and van Loon, 2009).

The aim of this study was to assess the reproducibility of the measurement of contractile properties by electrically elicited contractions over two testing sessions, spaced by 2 weeks.

Methods

Participants

Nine individuals (8 men and 1 woman) participated in the study. The study was approval by the ethical committee of Manchester Metropolitan University and was performed in accordance with the Helsinki Declaration. Descriptive data of the subjects is presented in table 3. All subjects were healthy and were recreationally active as assessed with health and physical activity questionnaires (Baecke et al., 1982).

Exclusion criteria were known cardiovascular, respiratory conditions, neuromuscular diseases and lower limb injuries.

Table 3. Characteristics (mean ± SD) of participants.

	Age (yrs)	Height (m)	Mass (Kg)	BMI	Body Fat %
N = 9	27.6±3.7	178±5	84.5±7.2	26.8±2.9	18.6±8.6

Procedure

Isometric knee extensions were performed with the right leg on a custom made isometric testing dynamometer (Designed by the Department on Physical and Medical Technology, VU University, Amsterdam, The Netherlands). Force signals were recorded via a customised Labview (National Instruments Corporation, Texas, USA) and Matlab software (Matlab, the Mathwork Inc, S Natik, MA, USA). All procedures were explained to the participants, emphasising the requirement to stay relaxed during electrically stimulated contractions and only to voluntary contract when instructed. The patients were seated on the dynamometer with a knee angle of 90° (Full extension being 0°) and 85° hip flexion (Supine being 0°). The lower leg of the participants was securely fastened to the force transducer, 2 cm above the ankle malleolus. The hip joint was held in place firmly via a strap. Prior to procedures participants were familiarised to the knee extension exercise, initially with three contractions around 50% of maximal effort lasting 3 seconds, followed by two further contractions around 80% maximal effort. A two-minute rest was given prior to a maximal voluntary contraction sustaining for around 3 seconds. Two or more maximal effort contractions were performed until the two highest values were within 10%, with the highest value taken as MVC. Verbal reinforcement and visual feedback were conveyed during the testing.

Voluntary activation, the ability to voluntarily activate the knee extensor muscles during an MVC was assessed with the interpolated twitch technique (Wust et al., 2008, Rutherford et al., 1986a, Van Leeuwen et al., 2012). Thereto, stimulation electrodes (AmericanImex, CA, USA) were placed on the proximal and distal heads of the quadriceps femoris muscles. Muscles were stimulated with 400 V pulses with a width of 200 μs (Digitimer DS7AH, Herts, UK). Before the test the current was increased so that a stimulation with two pulses separated by 10 ms known as a “doublet” elicited ≥

30% MVC. During the test, the doublet was followed by an MVC and at the peak of the MVC another doublet was administered. The voluntary activation (VA) percentage was calculated as:

$VA = 100 * (1 – t/T)$

Where T is the value of the resting doublet and t is the amplitude of the superimposed doublet (McPhee et al., 2014, Wust et al., 2008).

To construct the force frequency the muscle was stimulated with 2-second tetani (1, 10, 15, 20, 30, 50 and 100 Hz) in a random order with a 60 second rest in between. The maximal force at each frequency was expressed as a percentage of the force evoked at 100 Hz (Figure 1). Contraction rate (delta force/ delta time, max), normalised contraction rate (contraction rate/peak force), relaxation rate (delta force/delta time, min) and normalised relaxation rate (relaxation rate/peak force) were calculated from the 100Hz contraction (Degens et al., 2005, Wust et al., 2008).

To determine the fatigue resistance of the muscle the muscle was stimulated with 30-Hz pulse trains 1 s on 1 s off for 4 min. The resistance to fatigue was expressed as a fatigue index, which was calculated the 30 Hz force of the last contraction (Fend) as a fraction of the 30 Hz force of the first contraction (Fstart) fatigue index = Fend/Fstart (McPhee et al., 2014, Degens et al., 2005)

Statistical Analysis

A paired samples T-Test was used to assess differences between testing sessions using SPSS v21 (IBM, USA). Data are given as mean ± standard deviation (SD). R^2 values were calculated and differences between groups were considered significant at $P<0.05$. Moreover, the test – re-test variability was assessed for the entire group using pooled coefficient of variation (CVp), which was calculated as the SD of the differences as proportion of the mean.

$CVp = \sqrt{((\sum CV_i^2)/n)}$

(Gerrits et al., 2001)

Results

Table 4. Descriptive statistics and reproducibility of contractile properties of human quadriceps muscle in healthy young subjects.

	Test	Mean ± SD	CVp	R^2	P
MVC (Nm)	Pre	836±110	0.07	0.61	0.317
	Post	811±101			
FI	Pre	0.52±0.10	0.08	0.71	0.441
	Post	0.53±0.08			
VA (%)	Pre	85.7±11.5	0.06	0.69	0.362
	Post	83.6±10.5			
dF/dt Max 100Hz	Pre	15.8±0.9	0.08	0.890	0.394
	Post	16.4±1.5			
dF/dt Min 100Hz	Pre	-14.3±1.2	0.13	0.100	0.832
	Post	-13.9±1.7			

MVC: maximal voluntary contraction, FI: fatigue index, VA: voluntary activation, dF/dt max: most force rising ratio, dF/dt min: most force relaxation ratio, CVp: pooled coefficient of variation.

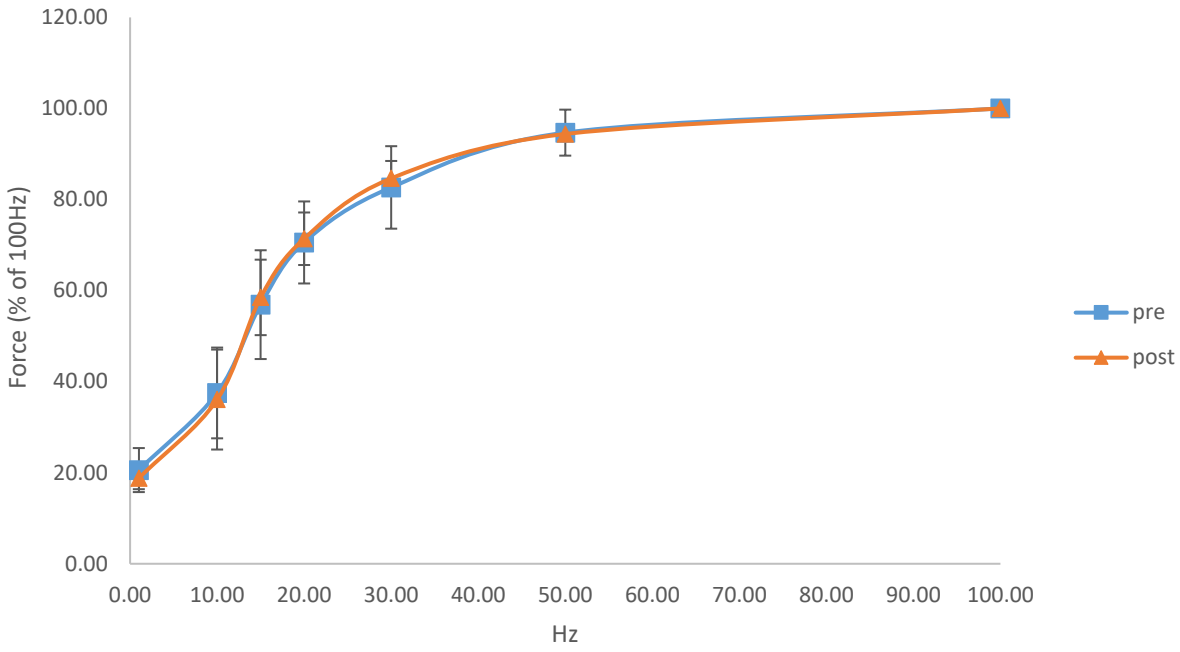

***Figure 5**. Force frequency repeatability relationship of contractile properties of human quadriceps muscles in healthy young subjects.*

Nine participants completed experimental tests for both testing days, where 6 also completed repeat tests for the force frequency relationship, and rates of force rise and relaxation. Table 4 shows that the different contractile properties measured in the first test correlated well with those in the second test (all $R^2 > 0.6$), and did not differ significantly. Figure 5 illustrates the excellent level of agreement between test sessions for the force frequency relationship. All bar the maximal rate of relaxation returned CVp's less than 0.09

Discussion

The main finding of the present study is that the contractile properties determined with electrically elicited contractions are highly reproducible in a period as long as 2 weeks, the coefficient of variation was less than 9% for all parameters, except the relaxation rate.

The coefficient of variation in our study for the determination of contractile properties with electrically evoked contractions was lower than that reported previously (Gerrits et al., 2001, Brass et al., 1996, Chan et al., 1999, Eerbeek and Kernell, 1991). Some of the variability in the test-retest measurements could be due to the incomplete

stimulation of the muscle via the surface electrodes which leaves some room for voluntary contractions interfering with the measurements (Gerrits et al., 2001).

It must be considered that some of the variation could arise from small differences in experimental environment, changes in the condition of the muscle due to previous activity, changes in dietary or hydration state. It is also possible that different fibres were recruited during the two sessions, though care was taken to position the electrodes at exactly the same locations on the muscle and use a current to elicit the same absolute force. In theory, muscles may have adapted to some training regime that was initiated, but we asked our participants to maintain their normal habitual activity levels, therefore we assume no changes.

In conclusion the current study confirms that in young healthy subjects the contractile properties of the quadriceps muscle can be determined with electrically evoked contractions via surface electrodes to a high level of reproducibility.